Heredity beyond the selfish gene: the Inclusive Evolutionary Synthesis

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For the last 20 years, an increasing number of scientists from various disciplines of biology have been calling for the modernisation of the Modern Synthesis of Evolution (i.e. the canonical vision of evolution), because of fascinating discoveries from all fields of biology seemed to challenge it.

However, to build such a new synthesis, we need more than simply calling for it. We need to integrate all recent discoveries from a wide variety of fields of biology (development, genetics, epigenetics, physiology, molecular, cell and medical sciences, as well as ecology and evolution) into a general and clearly defined new framework. This is what this scholarly and transdisciplinary book aims at doing. Beyond the mere statement of intent of tenants of the new synthesis, the book proposes a general framework (the Inclusive Evolutionary Synthesis) integrating all recent discoveries into a coherent and broader synthesis accounting for all the various forms of selfish replicating entities that participate to inheritance. In this sense, this book can be seen as a follow-up (or generalisation) of *Richard Dawkins*' famous Selfish Gene.

The Modern Synthesis emerged from the merging of two independent disciplines that largely ignored each other for decades, one dealing with natural selection and the other with genetics. Darwinians studied natural selection as the engine of evolution, while geneticists and biometricians studied Mendel's laws. The compatibility between Mendel and Darwin was later shown, fostering the emergence of the Modern Synthesis from the 1940s to the 1960s. Interestingly, these two initial disciplines belonged to *evolutionary biology* as opposed to what he called *functional biology* that studies mechanisms occurring within an individual organism. The Inclusive Evolutionary Synthesis ambitions to integrate facts and concepts of both functional and evolutionary biology into a single framework integrating all discoveries from their various sub-disciplines.

A major input of Darwin (and followers) was in specifying that evolution by natural selection (or drift) is a direct by-product of parent-offspring resemblance that constitutes the keystone of biology. Thus, to build the new synthesis the author focuses on heredity and mechanisms of transmitted resemblance (i.e. inheritance) as a major concept allowing the merging of evolutionary with functional biology.

Far from challenging the Modern Synthesis, the Inclusive Evolutionary Synthesis builds on it as a solid foundation, and enriches our understanding of evolution, hence increasing our predicting capacity.

This didactic book is written for three audiences, scientists specialized in evolution, students of biology, as well as a public of enthusiasts eager to understand life. A glossary defines the major terms and concepts of the evolutionary science's jargon.

Abstract

An ubiquitous fact of life is that offspring resemble their parents in many characteristics. Our everyday language is full of expressions based on family resemblances, which we call heredity. The prevailing view is that heredity results mainly, if not exclusively, from the transfer of genes from parents to offspring. But is this really the case? What exactly is meant by genes? Why is this parent-offspring resemblance so important? And how might answering these questions change in our everyday lives?

In this book, I reveal the extent to which biology is undergoing a rapid change, sometimes called a revolution, starting from the currently widely accepted "Modern Synthesis of Evolution"[1] towards a broader framework. In the first part, I present relevant aspects of the classical view of inheritance and evolution according to the mainstream vision of evolution usually called the Modern Synthesis, which fused population genetics with Darwinism. A milestones in the history of the Modern Synthesis was the publication of *Richard Dawkins*' book "The Selfish Gene"[2], which made the current view of evolution accessible to all. This book may be seen as an update of "The selfish gene". In the second part, I detail a selection of striking recent discoveries showing how, after having brought about considerable progress in biology for more than 70 years, the Modern Synthesis' view of evolution has reached its limits and now needs to be modernised. I explain how many astonishing discoveries made in all areas of biology since 2000 show that the sole transmission of genes is not sufficient to explain the complexity and diversity of life. In the third part I propose a new conceptual framework allowing a transition towards an assumed maturity. This new framework that I call the "Inclusive Evolutionary Synthesis" (or IES), builds on the highly successful Modern Synthesis of Evolution as a solid foundation. Far from rejecting the Modern Synthesis, the IES thus generalises and broadens it in order to accommodate all recent discoveries by integrating all currently known forms of inheritance into a single synthesis with a greater explanatory power. In doing so, I explain how natural selection or drift act on, and thus cause the evolution of, any information, whether genetic or not, as soon as it is transmitted across generations.

Hence, inheritance and evolution appear as a multidimensional process that cannot be fully understood if we reduce it to its sole genetic dimension. I, along with many others, defend the idea that evolution is the result of a series of inheritance processes of a genetic, epigenetic, cytoplasmic, cellular, cultural, ecological nature, which may involve the transmission of microbes, or even that of proteins in specific states. Each of these inheritance systems works at a specific pace allowing organisms to adapt to environmental variation at all timescales. Through their interactions, these different processes shape evolution. Finally, in the fourth part, I show what this new vision of inheritance and evolution changes in various domains of evolutionary, medical and conservation sciences, with the hope of convincing the reader that the plurality of inheritance systems has the potential to change considerably decisions in our everyday life.

Thus, this book speaks about our deep nature, as well as about the philosophical and societal issues related to the great environmental challenges we are currently facing. Recent discoveries and events that have affected humanity show that such a goal must become a major ambition of our societies. The future of all living beings on planet Earth depends on it.

Foreword

After kindly agreeing to read the entire manuscript of this book, *Richard Dawkins*, author of "The Selfish Gene" and many other works, sent me numerous interesting and constructive comments, along with a letter which (with his permission) I quote here in detail, as it sheds a central light on my argument in this book. His exact words were "…, *if you'd like to quote (at any length) my letter to you on Chinese Whispers, you are entirely welcome to do so, provided you make it clear that it is from a letter to you and not a piece of polished prose written for publication*".

This letter, received on 15 February 2022, focused on two important and related points.

The first point concerned the necessity for a piece of heritable information to persist for an indefinite number of generations (even if its transmission fidelity is imperfect), for it to be comparable to nucleotidic information. To spell out his point Richard Dawkins developed the following example:

> "Here's a memetic example. The game of Chinese Whispers (American "Telephone"). Line of twenty children who all speak the same language, say English. Whisper a line of poetry to first child who whispers it to the second child, and so on. Probably by the time it reaches the 20th child it will be changed, but it may survive.
>
> If the poetry survives, it does so despite being repeated in different accents, Yorkshire, Scottish, Irish, American, Australian etc. But as long as the children all understand the same language it can survive through the *normalization* process of each child recognizing each word as a familiar word.
>
> But now suppose the poetry is in a language the children do not know, say Basque. It's obvious what will happen. Repeating the sounds phonetically with no normalization, the distortion by the twentieth child will be almost total.
>
> Now, here's the key point. Suppose that, in the two experiments, we secretly record each child's whispering. So we have English Recording 1, 2… 20: E1, E2… E20. And we have Basque Recordings B1, B2... B20.
>
> Now take naïve observers and present them with B1 to B20 in random order and ask them to rearrange them in their correct order. I assert that they will have no difficulty doing so, at least approximately. B(n) will be a recognizable distortion of B(n-1), and so on down the line to B20, which will be a recognizable distortion of B19. B17 will sound much more like B16 than it sounds like B5. And so on. There will be a recognizable order of distortion which will correspond to the chronological order.
>
> The E series will yield a very different result. Assuming that the poetry survives, it will be impossible for the naïve observers to rank the E recordings in the order that they occurred. Every E may be a distortion of its immediate predecessor because of different regional accents, but E(n) will no more resemble E(n-1) than it resembles E1 or E 20 or any of the Es. If child 15 and child 7 both happen to be from Liverpool, E15 and E7 will resemble each other, being pronounced with a Scouse accent, whereas E14 may be Scottish and E6 may be Devon.
>
> If the poetry does not survive to E20, it will be because of a discrete mutation somewhere along the line. For example, E9 may be different from E8 because the child changes one English word to another English word. The mutation will then persist from E10 onwards (until another mutation occurs, and so on). The naïve observers will easily classify the recordings as pre E9 and post E9, but they won't be able to rank them within each of these two categories.
>
> Conclusion to the analogy. Words in a familiar language are Evolutionarily Significant Replicators. Words in an unknown language, in this case Basque, are not.
>
> An Evolutionarily Significant Replicator, ESR, must behave like the E series, not like the B series. DNA is an excellent ESR. It survives with perfect fidelity until a discrete mutation changes it, whereupon the changed version will survive with perfect fidelity until it changes again. The other important barrier to its survival is, of course, natural selection. And that is the evolutionarily important effect. But natural selection has no chance to work, if the putative replicator isn't going to survive ANYWAY because it is like a Basque word in a line of English-speaking children.
>
> DNA may not be the only ESR. Etienne, you are right to be open to generalising "Replicator" more widely. And you are right that memes are good candidates, to the extent that they behave like the E series. As words (of a familiar language) do, because of the normalizing effect, at least over a few centuries (back to Chaucer we have problems!). Normalization of this kind may not be

> the only reason for long-term fidelity, but long-term fidelity must be there for the replicator to be evolutionarily significant. Long term as measured on the time-scale set by the selection pressure."

I could not have said it better. We will see that this generalisation probably does not only apply to the cultural replicator and can probably also concern the epigenetic replicator. But the point is that there are probably many types of replicating entities alongside the genetic replicator.

Dawkins also correctly took issue with my description of replicators to include information that is transmitted across generations (and which therefore contributes to parent-offspring resemblance) but which survives only for a limited number of generations. I have therefore introduced the notion of pseudo-replicator and drawn attention to the fact that non-genetic inheritance systems may lie somewhere on a scale from pseudo- to true replicators. I am grateful to him for these important points.

To sum up, my main argument in this book is not that all non-genetic inheritance systems qualify as replicators. Rather it is the claim that alongside the sequencic replicator, there are replicator-like processes acting at shorter timescales, participating in inheritance and selection, and thus affecting the evolutionary fate of populations. Furthermore, the convergences in my discussion with Richard Dawkins perfectly illustrate the fact that far from challenging the Modern Synthesis of Evolution, the Inclusive Evolutionary Synthesis that I hope to establish in this book in fact is just a generalisation, some might say a refinement, of the Modern synthesis. In other words, the Inclusive Evolutionary Synthesis is built on the solid basis of the Modern Synthesis. This is a central credo of this book.

Toulouse 8 March 2022

Teaser

The trauma of the heredity of traumatic experiences

Can you imagine that a smell that everyone around you finds harmless could cause you to react with fear, simply because one of your grandparents had a bad experience with that smell many years before you were born? If modern psychiatry has familiarized us with the idea that our own past can haunt us, we now realize that the past of our ancestors can also affect us. This book deals with such phenomena, which are now proving to be far more numerous and stranger than we suspected only twenty years ago.

I did not choose the example of a frightening smell randomly — quite the contrary. After all, this is exactly what was documented by *Brian Dias* and *Kerry Ressler* in 2014[3]. This article caused quite a stir. It showed that mice conditioned to associate a benign odour with something unpleasant, not only remember it for the rest of their lives, but their offspring and grand offspring 'remember' it even though they were never conditioned to that odour and never interacted with their parents or grandparents. More precisely, offspring and grand offspring show an increased sensitivity to that specific smell, potentially leading them to develop the same fear of that smell as their parents or grandparents did. How is such a thing possible? How can a learned fear be passed on to offspring in the absence of any intergenerational contact? These are some of the many questions raised by that study.

For my part, I had heard about this article in early December 2013 from Eva Jablonka whom I met in a workshop in Giff sur Yvette south of Paris. Eva is one of the pioneers in challenging the classical view of *heredity* (see Glossary), long before I became interested in the issue myself. Although I was not shocked by this result because I had been predicting it, it didn't stop me from experiencing what I call the "aesthetic thrill" (my phrase to describe the intense pleasure brought by a particularly stimulating idea). I was enchanted by their result.

That article's high impact had several effects. First it rigorously demonstrated that traumatic experiences can affect the development of offspring over several generations, leading them to behave like their parents or grandparents. Second, it provided compelling experimental evidence on the molecular mechanisms of *inheritance* (see Glossary) of traits developed during life[4]. Third, it demonstrated that such transmission actually occurs through both male and female gametes of traumatized fathers and mothers. This article thus reopened the debate on the transmission of acquired traits, a form of heredity that is widely considered as "heretical"[5]. This is why it was accompanied by a commentary in the same issue of *Nature Neuroscience* by *Moshe Szyf* of McGill University in Montreal with the explicit title: "*Lamarck revisited: epigenetic inheritance of ancestral odor fear conditioning* that explained how these remarkable results raised a number of major biological questions, which we now had to address head on[6].

Can we talk of heredity in this case?

A first question is whether this transmission of reactions to environmental factors belongs to heredity. But, what is heredity exactly? We have all been taught that heredity can be reduced to the transmission of *genes* (see Glossary). But is this really the case? Moreover, the unusual result outlined above also raises the question of why heredity is so important in biology. These are major questions that I will address in this book.

A new context that leads to reflection

We will detail this specific example in **Chapter 8**. But we can see already that this kind of result seems at odds with the dominant view of heredity and evolution as it is currently taught. But does it really challenge our view of heredity and evolution? We will see that it is not easy to answer this question, but that such discoveries force us to rethink our vision of life. It is exactly the aim of this book to illustrate how the modifications of the mechanisms of heredity imposed by recent discoveries are profoundly changing the way in which we understand life and, in particular, the way in which we approach the human species itself.

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More generally, since the end of the 1990s, as a result of a series of discoveries of the type I have just sketched above, ideas about heredity have been undergoing a mini revolution, in which I have had the fantastic good fortune to participate. It is therefore the right time to describe how the current developments in our conceptions of heredity are shaking up our vision of life, with repercussions throughout biology, from medicine to ecology, evolution and conservation biology, but also in the humanities and philosophy. My aim is to explain the fundamental reasons for these ongoing changes. Without calling into question your general ideas on the

central role of genes, you will see that the discoveries currently being made considerably enrich and diversify the mechanisms of heredity and, beyond that, the very functioning of living beings.

I will therefore accompany you towards a better understanding of heredity and what all this means for us, citizens of the world. The idea is to do this in a simple and exciting way. I will start with three chapters summarizing the mainstream conception of inheritance. Then, in nine chapters, I will describe the recent discoveries that call for a modernization of that conception. In the third part, I will reconcile and integrate the different views of inheritance, often described as incompatible, within the Inclusive Evolutionary Synthesis (or IES). Finally, in the fourth part I will discuss the extent to which these conceptual changes actually affect evolution, as well as everyday medical and conservation actions, and also philosophy.

This book is written for three audiences, researchers and students in biology and evolution, but also for a public of enthusiasts who are eager to understand life, potentially to better manage nature. To make reading easier, a glossary is provided at the end of the book, to define the major terms and concepts of the scientific jargon. The first appearance of each of these terms it will be in italic and followed by "(see Glossary)" to indicate that a definition can be found there. Finally, you should know that there is a simplified version of this book in French —published in May 2021 by humenSciences[7]— aimed at people who are unfamiliar with biology and evolution. This simplified text can constitute an introduction for the layman.

Part I

Heredity according to the Modern Synthesis of Evolution

Chapter 1
What is heredity?

On a cool May morning in 1979, I was walking in Quiberon (in Brittany) with my new-born son in a kangaroo bag on my chest, hidden under my closed coat and he was wearing a cap to protect him from the cold, his face turned towards my chest. While no one could really see my son's face, I nonetheless got a "A chip off the old block!" from one of my in-laws' neighbours whom I passed that morning. Without being the least bit aware of it, she was trying to make me feel secure in the fact that the sperm that had produced this child was really mine. Her statement was based on a major biological fact, namely that children strongly resemble their parents. In fact, when they were babies, my three children, boys and girls, looked so much alike that when I look at pictures of them as babies, it is the details around them that let me know which one they are. Then, of course, they became very different looking, and the experience of watching them being born, growing up, learning to walk, then talk, think for themselves and become independent adults is one of the most fascinating life experiences. There is something really 'magic' about the incredible ability of life to re-produce an autonomous adult from a single cell, itself the result of the fusion of an egg and a sperm cell.

Reproduction has always fascinated humans, leading people like me to dedicate our lives to biology. The more we understand this phenomenon, the more we are struck by the incredible refinement of the many mechanisms that interact during reproduction and development. It is this capacity that still distinguishes living from non-living entities: that which is alive can reproduce itself autonomously[8].

One of the primary characteristics of reproduction that has long occupied researchers is parent-offspring resemblance, which lies at the very heart of the concept of *heredity* (see Glossary). This major property is all the more striking in that it concerns all types of traits of the *phenotype* (see Glossary), including morphological and physiological traits such as the size or colour of various body parts, and behavioural traits, such as diet or the ability to run fast, play tennis, attract sexual partners, think etc. As my neighbour from Quiberon noted, this resemblance is an implicit fact in everyday life.

However, if today we have clearly assimilated parent-offspring resemblance as an incontrovertible fact, it was not always the case in the past. In ancient times up to the medieval period it was often believed that parents of very different species could produce mosaic hybrids, made up of parts inherited from one parent and other parts received from the other parent. Mythology is full of such chimeras. Nevertheless, the study of reproduction and parent-offspring resemblance has been central to the emergence of the science we now call biology (etymologically: bios = life, logos = discourse, and by extension science). Today more than ever, the question of the mechanisms and processes that generate resemblance between parents and offspring remains at the heart of biology.

The fantastic discovery of DNA

When I was a student in the 1970s, the discovery of DNA in the 1950s and of the genetic code in the 1960s was less than 20 years old. It was an absolutely fascinating discovery and I consider it as the greatest discovery of the 20th century in any science. But we will see that this discovery has fascinated us so much that it has somehow blinded us to a series of phenomena suggesting that inheritance does not boil down to the sole transmission of the DNA sequence.

In fact, I am a pure product of the discovery of DNA because all the teaching I received at university was centred on this major breakthrough, and that was a very good thing. At university I was taught about discoveries that were still hot off the research stove. It is this research-teaching connection that makes universities unique. In particular, I took a genetics module at the *Université Pierre et Marie Curie* in 1974-75[9]. Then, in the years 1990-2005, I was part of a research laboratory that included a team of population geneticists who, by organising numerous seminars, strongly influenced and educated me in this field. I have to thank them for that because they gave me the basis to go farther.

What do we mean by heredity?

In order to proceed, we need to know what exactly is meant by heredity. What is heredity and why is this concept so central to biology and evolution?

Heredity, a question of parent-offspring resemblance

As we have seen above, heredity is about *patterns of parent-offspring resemblance*[10]. Such resemblance refers first of all to the fact that children belong to the same *species* (see Glossary) as their parents. This is what the expression "dogs don't make cats" means. Today, this fact is transformed into a postulate to define a species as the set of individuals that can reproduce with each other. Second, within-family resemblance resulting from parents passing on their own characteristics is more interesting. For example, we would say "this child is tall, which is normal because his/her parents are tall", or "big cats make kittens that on average will become bigger cats than the offspring of small cats". This parent-offspring resemblance therefore concerns the variation that exists within a population. Such variation can concern all traits, such as skin colour, eyes, dander (hair, feathers, scales), size, and behavioural traits etc. It is parent-offspring resemblance that maintains intraspecific variation, which is key for the evolution of life, and the fact that differences within a population are passed on to offspring is classically called *heritability* (see Glossary)[11]. Heritability, which can be defined as *the heredity of differences* quantifies the degree of parent-offspring resemblance.

Heredity and inheritance mechanisms

The word heredity is often used to describe two important aspects of reproduction. First, it describes the fact that offspring resemble their parents. This understanding therefore focuses on *patterns of parent-offspring resemblance*, with heritability quantifying the statistical degree of resemblance between parents and their offspring. It quantifies how much of the phenotypic variation (the fact that we are all different) is passed on across generations *by genetic means*[12]. Second, the word heredity is also used to refer to the *mechanisms* that produce this resemblance. We will see that these mechanisms involve the transmission of a wide variety of biological *information* (see Glossary) from parents to offspring. In this book, I will use the term *'inheritance'* to refer to this second meaning, while keeping the term heredity to refer to patterns of parent-offspring resemblance.

Pathways of heredity

We shall see later in this chapter that, from an evolutionary perspective, the fact that offspring resemble their parents is necessary to allow the *evolution* (see Glossary). And this is true even if this resemblance persists over only one generation, in which case the evolutionary impact will probably be low, but nonzero.

Researchers studying the mechanisms of this resemblance have emphasized several important aspects. First, for parent-offspring resemblance to be truly indicative of heredity and directly affect evolution, the resemblance must result from a transfer of some information between parent and offspring (Table 1). A parent-offspring resemblance over one generation may sometimes simply be due to the fact that the offspring and their parents were *simultaneously* (albeit at different ages) subjected to the same environmental stress. In such cases, the resemblance does not reveal heredity but only *simultaneous exposure* (see Glossary) to the same *environment* (see Glossary). To clarify the various possibilities, let us focus on the causal pathways involved in parent-offspring resemblance (Table 1). It is important to distinguish between cases where transmission involves the germline (i.e. either through eggs or sperm or both). Authors have therefore given different names depending on the type of argument demonstrating parent-offspring resemblance (Table 1).

- *Simultaneous exposure effects* are cases in which a given environmental factor simultaneously affects gene expression of both the parents and their germ cells, i.e. their future offspring. Hence, resemblance only arises from the fact that the two generations were exposed to the same environmental effect, albeit at very different stages of their lives, but simultaneously. Such cases do not reveal heredity (Table 1). Note that if the parent undergoing the environmental stress is a pregnant female, then the simultaneous exposure can affect 3 generations, the mother, the developing embryo and its already differentiated germ cells[13].

Table 1: When does resemblance reveal heredity? We will see examples in the second part of this book.

Cause of resemblance *Type of mechanism of parent-offspring resemblance*	Does parental trait affect that of offspring	Transmission fidelity across generations	Does transmission involve gametes?	Can we speak of heredity?
Simultaneous exposure	No, just a co-occurrence	None	No	No
Transmission via the environment	Not applicable	Weak	No	Yes
Epigenetic transmission	Not applicable	Moderate	Yes or No	Yes
Genetic transmission	Not applicable	Strong	Yes	Yes

- Cases where resemblance is demonstrated between the manipulated generation (usually called F0) and its immediate descendants (called F1) are referred to as *intergenerational effects*.
- Experiments showing that the resemblance persists at least until F2 (the grand offspring) are termed *multigenerational effects*.
- When the resemblance persists beyond F2, we speak of *transgenerational effects*.

This is why all the examples in the second part of this book show that the effect persists at least until the second generation, because this is the only way to be able to claim that the study trait is inherited in experiments in which manipulated females are not pregnant.

Once we have defined heredity, it is worth asking why it is such a central concept in biology. The primary reason for its importance lies in the very process of natural selection, a concept whose theoretical foundations were established independently and simultaneously by *Charles Darwin* and *Alfred Russel Wallace*[14].

Why is heredity so important?

A bit of history

Let us take this opportunity to remind ourselves that, contrary to what is often heard, *Charles Darwin* did not invent the concept of evolution. *Charles Darwin*'s grandfather, *Erasmus Darwin*, was already a transformist (i.e. an evolutionist). It was an idea that had been around for several decades. It is usually accepted that the first scientific formalisation of the idea that species change over generations was made *by Jean-Baptiste Lamarck* in 1809 (the year *Charles Darwin* was born) in his book entitled "*Philosophie zoologique*", which was the written version of the lecture he gave in 1800 when he took up the chair of zoology at the Paris Natural History Museum. So, at the time of *Darwin's birth*, the major advance of accepting that species can evolve over time was already a scientific topic. However, *Lamarck*'s work was missing an important ingredient, for while he proposed that species change over generations, he had not actually proposed a mechanism that could produce such transformations. It was *Darwin* and *Wallace* who's contribution was in proposing the mechanism of natural selection.

Heredity, evolution and natural selection

What is evolution?

Historically, one of the strongest arguments in favour of the existence of what is now called evolution comes from the fact that among the fossils found in sediments, which can sometimes be thousands of metres thick, most fossil species are found in a given place only in certain contiguous geological strata and in no other strata. However, in deeper (and therefore older) geological strata one finds species that are very similar to them; and in shallower (and therefore more recent) strata one finds other fossils that have affinities with the concerned species. It would seem, therefore, that species have changed gradually over time in an unbroken series of successive species.

This is how the idea of what was first called 'transformism' and what we now call evolution came about[15]. However, this now widely accepted idea initially raised difficulties because according to biblical texts it was believed that the world, and in particular the species, were created by God immutably. The very idea of evolution was then widely considered to be deeply contrary to religion. Today, there are so many concrete facts demonstrating the existence of evolution that the subject is no longer a matter of scientific debate. After all, the very rapid appearance of new variants of Sars-Covid-2 that rapidly replace each other shows how everything is constantly changing and that all populations of all living organisms are inexorably evolving.

Today, studying evolution means studying the processes that lead species to change over time. This is the aim of my laboratory in Toulouse, which is called "*Évolution & Diversité Biologique*" or EDB.

Implicitly we all learned that changes in phenotype are underpinned by changes in the sequences of the DNA molecules (i.e. mutations) that may or may not be favoured by natural selection and that may accumulate over geological time. My goal in this book is not to claim that this relation between phenotypic change and genetic change is wrong, but rather that it is incomplete.

Natural selection

Let us now return to natural selection. Unlike their contemporaries, *Wallace* and *Darwin* were interested in the ubiquity of within population variation. It was this interest that led them to the mechanism of natural selection. Natural selection is a process, i.e. a chain of phenomena linked by causal relationships. This process is inexorably set in motion when two conditions are met and will lead to evolution if a third condition is met[16]:

a) *There must be variation among individuals within a* population, otherwise there is no basis for selection. Variation is the raw material of evolution. As already mentioned, this condition is almost always met because intra-specific variation is everywhere.

b) *There must be a persistent relationship between the values of the study trait and the ability of individuals carrying it to produce offspring*, a capacity known as *fitness* (see Glossary). This second condition corresponds to the selection pressure. For example, it could be the tallest individuals that have the highest fitness. The proportion of their offspring will therefore increase in the population over generations, pending on the fact that this advantage to the taller individuals persists over generations.
c) For this selection pressure to cause the trait to evolve (in the above example, to cause an increase in the size of the concerned trait) over the generations, *the differences must be inherited*. In other words, the offspring must be more similar to their parents than to no-relatives, i.e. the trait must be *inclusively heritable* (see Glossary).

To illustrate the importance of this last condition for evolution, let's take the example of a farmer living off the milk of his cows. For as long as animal husbandry has existed, our ancestors have favoured the cows that produce more milk, allowing them to reproduce, while those that produced little milk went to the slaughterhouse without reproducing and were therefore counter-selected. By only allowing cows that produce a lot of milk to reproduce, farmers had thus artificially selected for high-milking cows. But this selection could only lead to an increase in the number of litres of milk per cow over generations if high-milking cows had daughters who themselves produced more milk than the daughters of low-milking cows. If this were not the case, despite having selected heavily for generations for high-milking cows, farmers would not have been able to induce a significant increase in milk production per cow. The milking production per cow would not have evolved.

We now see why heredity is so central to biology. *It is the heredity of differences that allows the selection pressures exerted by the environment (whether natural or artificial) to produce evolution*. Without this heredity of differences, there is no evolution. Another way to say it is to say that "*Evolution by natural selection provides the bridge between mechanisms and purpose*"[17]. It is the genius of *Darwin* and *Wallace* to have understood this and to have added this pillar to the conceptual edifice that is the field of evolution today.

The debate on the mechanisms of heredity

A large part of the history of biology has been structured around the question of the mechanisms that produce parent-offspring resemblance, i.e. heredity, in relation with the sources of variation among individuals. Why do individuals within a population, or even among siblings, differ so much from each other? The scientific debate on these issues has never really stopped[18].

However, while the end of the 20th century saw the triumph of a very reductive vision of genetics and heredity so that this question seemed to have been settled for good, by a strange reversal of history, this debate has undergone a revival since the beginning of the 21st century. This revival resulted from a series of major discoveries which, once put into perspective, force us to rethink our conception of heredity. Beyond the sole theoretical consequences, the recent developments we are about to see have immediate and profound practical consequences in medicine and in the field of ecology, evolution and conservation biology.

I have been lucky enough to be one of the actors in this ongoing quiet revolution, and it is why I am writing this book. More generally, beyond the fact that I hope to convince you that the current rapid evolution of our conception of heredity is quite exciting in itself, all the discussions I have with my colleagues lead me to believe that this renewal is profoundly changing our understanding of life, with deep ramifications in all fields of biology and philosophy. But first, it is important to understand the reasons that have led to the reduction of heredity to the simple transmission of a genetic programme encoded in the DNA sequence.

We should not overestimate the role of the DNA sequence

DNA an essential biological memory molecule

Like any biologist trained in the 1970s and beyond, I learned that heredity can be reduced to the sole transmission of genes from parents to offspring. The general idea is that in any sexually reproducing organisms individuals result from the fusion of two cells, one from the mother (called the female gamete or ovum) and one from the father (called the male gamete or sperm). As each of these gametes containing a complete set of *chromosomes* (see Glossary), the resulting egg therefore contains two versions of the species genetic information, i.e. two sets of chromosomes, one set from the mother and one set from the father. Such organisms, which are called *diploid* (see Glossary), constitute the vast majority of living beings that we see around us. For example, each of human being has received 23 different pairs of chromosomes from their two parents. Similarly, in asexually reproducing organisms, every individual receives a complete set of chromosomes from their single parent, but this does not change much for the rest of this book. This transfer from parents to offspring occurs in every generation and has been going on uninterrupted since the origin of life on Earth some three and a half billion years ago[19]!

What all the lectures I attended have told me, is that the carrier of heredity is (implicitly, 'is' here could be replaced by 'reduces to') the Deoxyribonucleic Acid molecule, i.e. the famous DNA molecule that plays such an important role in criminology today. DNA is shaped like a ladder, with each rung being a pair of *nucleotides* (see Glossary). This ladder is not straight, but twists in a sort of infinite spiral staircase called the 'double helix'.

The properties of this molecule are remarkable. In short, the DNA molecule is a sort of polymer of a basic component called a nucleotide. Just as a train is made up of a long chain of coaches, DNA is made up of a very long chain of nucleotides (the entire DNA sequence of a humans contains 2x3.2 billion nucleotides). In this huge DNA train, there are only 4 types of coach (nucleotides), Adenine, Cytosine, Thymine, and Guanine (written A, C, T and G). Although it is never stated so clearly, according to the view I was taught, the intrinsic properties of this molecule lie in its sole nucleotidic sequence, which is written as CCATGGCTTAGCATGC over 3.2 billion characters in humans. To give an idea of what this represents, if I were to write the entire sequence of a human being as above, counting 4 letters per centimetre, this sequence would measure 19,200 km and would cover about 3.2 million pages of this book[20].

The discovery of the DNA structure by Watson, Crick, Wilkins and Franklin in 1953[21], made it clear that this colossal sequence of nucleotides constitutes an excellent substrate for encoding *information* (see Glossary). In effect, that discovery paved the way to the deciphering of the genetic code that explains how genetic information is somehow digitized in a base 4 system (involving the 4 letters ACTG, each standing for one of the four types of nucleotides), just like all the operations (text, image, music, etc.) performed by our computers, phones and tablets use binary information consisting only of sequences of 0s and 1s.

In essence, the genetic code explains how the cellular machinery can translate the DNA sequence into proteins, which themselves consist of a sequence of about 20 types of amino acids. The DNA sequence of any organism encodes the amino acid sequence of almost all the proteins needed for its life. Hence, a *gene* (see Glossary) can then be defined as the information carried by a portion of DNA, which encodes the amino acid sequence of a given protein.

Sequencic, or the view that reduces inheritance to the transmission of the DNA sequence

Thus, according to this view, all the information to make all the proteins necessary for an organism's life is encoded into its DNA sequence alone, so that by transmitting the entire DNA sequence to our descendants, we transmit all the information needed to reconstruct all the proteins and functions necessary for the development and functioning of a new individual organism. This is what I call the *'sequencic'* view of life (see Glossary)[22]. The important point is that this sequencic view of inheritance assumes *that the information necessary and sufficient for reconstructing an individual boils down to the encoding of the sequence of all the proteins that constitute this individual*.

As such, the functional scheme linking the DNA sequence to the protein sequence remains entirely correct. But we have been so collectively fascinated by this fantastic discovery, a fascination reinforced by the tremendous successes of molecular biology in the second half of the 20th century, that the idea has unfortunately taken hold that the information passed on from parents to offspring only involves the information encoded into the DNA sequence and no more. Our fascination for the DNA sequence has gradually reduced our conception of heredity to the transmission of this DNA sequence alone.

If you are not convinced that this is the dominant view, you can ask your friends or random people in the street how they would define a gene. I've been doing this for 30 years, several times a year, with my students at university. Almost everyone you ask will end up saying that a gene is a piece of DNA. Then ask them to be more specific about what this has to do with life, and therefore reproduction. You will inevitably end up with a sentence stating that this piece of DNA carries, through its nucleotidic sequence, information that is at the source of life. If you ask them if they think this fully explains heredity, they will assertively answer 'yes' (except for students who already know that I work on non-genetic inheritance).

This sequencic vision of heredity is very deterministic. According to this view, organisms are only the by-product of the interaction of their sequencic information (itself inherited from the parents according to very precise laws) with their environment. As a result, we are to a large extent determined by the sequencic information we inherited from our parents. We shall see how partial this view is, and how it ignores the many other forms of memory in living organisms that play a crucial role in heredity and therefore in evolution.

Proteins are the fundamental molecules of the cellular machinery

We can make a parallel with the study of protein functions to illustrate some of the issues raised by a purely sequencic vision of life. Proteins are essential to life and there are countless varieties in each organism, each with its own function(s). The study of their diversity and properties is called proteomics.

The properties of proteins depend primarily on their linear amino acid sequence, which, as we have seen, is directly encoded into the nucleotidic sequence of DNA[23]. However, it soon appeared that the amino acid

sequence of a protein alone is not sufficient to explain all its biological properties. This sequence (called the primary structure) constitutes a chain that winds in a helical fashion. This first winding is called the secondary structure of the protein. On an even larger scale, this helix folds again, giving the final three-dimensional shape of the protein, called the tertiary, or 3D structure. It is this 3D structure of proteins that gives them their biological function. This 3D structure depends of course on its sequence, but not solely on it.

If a protein is misfolded (due to the effects of its immediate environment), even though it has the correct amino acid sequence, it will no longer have the properties necessary for the proper functioning of the cells and the body. There are diseases that are caused by such misfolding of a particular protein. The best example is that of prion diseases where there is no genetic mutation, i.e. there is no difference in the DNA sequence between individuals expressing the disease and those not expressing the disease. This is the case with mad cow disease, for example, or scrapie in sheep, or Creutzfeldt-Jakob in humans. The changes responsible for these diseases are to be found in phenomena of a completely different nature, but which are nevertheless highly persistent and sometimes transmitted, and which involve interactions between proteins of the same sequence. The misfolded proteins serve as a template that transform efficient proteins from their initial functional to a non-functional configuration. This is how these diseases develop and how they can be transmitted, including through feeding with meat-and-bone meal.

Thus, the functional properties of proteins are only partially explained by their primary sequence, and other aspects independent of the amino acid sequence are also involved. In particular, some environmental effects such as heat shocks may foster changes in shape of proteins, and there are proteins, called chaperon molecule, whose biological function is to maintain other proteins in their functional shape. Since proteins' activities derive from their shape, which is only partially explained by their sequence, limiting ourselves to the study of their amino acid sequence would not provide us with a full understanding of their function. No biologist would dispute this major fact. And yet this is what we do all the time when we discuss genetics, we limit ourselves to the study of the DNA sequence; we usually ignore the question of the 3D shape taken by this molecule.

A parallel between the structure and function of proteins and those of DNA

What I have just said about proteins applies to the DNA molecule on a much larger scale. The functional and memory properties of the DNA go far beyond the information encoded into its nucleotidic sequence. This parallel has many different aspects, of which the following are three examples unfolding at very different physical and temporal scales.

- First of all, beyond the information encoded into the DNA sequence, the way that molecule is packaged within the chromosome in the cell nucleus carries further information. We can then speak of another type of molecular memory which takes the material form of the 3D structure of the DNA within the *chromatin* (see Glossary). This packaging involves many different proteins, some of which can be modified by small changes such as the addition of a radical derived from simple molecules like methyl or ethyl radicals. To simplify to the point of caricature, all this packaging protects the DNA molecule but also makes it accessible (known as euchromatin) or inaccessible (known as heterochromatin) for the expression of the genes present in the concerned part of the DNA molecule. The study of these incredibly complex and diverse processes falls within the field of *epigenetics* (see Glossary), a field that has been developing rapidly over the last 20 years and that will be the theme of **However,** before going into the description of these many striking examples, it is necessary to take the time to introduce a fascinating and rapidly growing field of organismal biology, that of epigenetics.
- The second fundamental memory property embedded in the tertiary structure of DNA is the indisputable fact that its 3D properties are themselves transmitted in parallel, but independently, to daughter cells during cell divisions (called mitosis). This is called mitotic epigenetic inheritance. There is a sophisticated molecular machinery during a cell division that copies the 3D structure of the DNA within the chromatin. This transmission property is at the heart of cell differentiation processes without which the development of multicellular organisms (which represent some 99% of the living organisms we perceive around us) would be totally anarchic.
- Finally, the third important property for our purposes is that some aspects of the tertiary structure of DNA within cell nuclei are also transmitted during sexual reproduction, thus contributing greatly to parent-offspring resemblance. This is an overlooked property of heredity for which I will develop many striking examples in the second part of this book. This third property is at the heart of the current debate on the sources of variation among individuals.

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The DNA in the chromatin is a kind of gigantic prion[24]

The importance of the 3D structure of DNA within chromatin is such that one can look at DNA and chromatin as a kind of gigantic prion because its configuration (*i*) affects its biological function, (*ii*) is transmitted very faithfully during mitosis, (*iii*) but also intergenerationally. We will talk briefly about prions again in the third part of this book. The idea in drawing a parallel between the function of proteins and that of DNA is to emphasise the formidable memory capacity of this molecule within chromatin, which goes far beyond the mere genetic encoding in its nucleotidic sequence.

A new definition of life

In the previous sections I have frequently spoken about biological information. It appears that the specificity of living beings lies in their capacity to collect, store, memorise, transmit, retrieve and use information about the *environment* (see Glossary). We could in fact redefine life around this concept of information: life is a memory machine about the *environment*[25]. To conclude this chapter, I will clarify briefly the meaning of 'information' and 'environment'.

Life is information

The concept of information in biology requires a definition. This is an extremely difficult question that would require an entire book on its own. But I offer here a pragmatic definition that we proposed with my colleague *Richard H. Wagner* in 2010[26]. We defined information as *any factor that can affect phenotypes* [the characteristics of an individual organism] *in a way that may influence their fitness* [i.e. their ability to survive and have offspring].

The capacity to gather, store and use information involves extremely varied and sophisticated mechanisms of memory that unfold at many different scales[27]. The lowest level of memory concerns the structure and configuration of biological molecules. Since these configurations are relatively stable, in the sense that a certain amount of energy must be supplied to change to another configuration, they constitute a first level of memory, known as molecular memory that can take various forms. For instance, the DNA molecule can carry a variety of information: over the very long term through its nucleotidic sequence, and on shorter scales through its 3D configuration, parts of which persist throughout an individual's life and can be passed on over many (sometimes hundreds or even thousands) generations. Other memory mechanisms exist at the cellular level, such as cytoplasmic memory[28]. Another form of memory is located at the level of a tissue (i.e. a coherent set of cells) or an organ (made of different tissues) within an organism. This is the case of the nervous system and in particular the brain, which harbours common sense memory. It is the one we summon at school, and it is the one I solicit from the readers of this book.

Environment and memory

I take the term environment in its broadest meaning, which includes all its abiotic (temperature, humidity, wind, reproduction sites, etc.) and biotic components (food resources, predators, parasites, social components such as sexual mates, competition, social habits) beyond the limits of an organism. Depending on the entities, it can be defined at different scales such as a cell within a tissue in an organism or an individual in its ecological and social environment.

Summary

Heredity concerns patterns of parent-offspring resemblance. It is central to biology because natural selection and evolution cannot occur without heredity. It is thus vital to study the mechanisms that produce this resemblance that involves the transmission of many kinds of information from parents to offspring. Living organisms can therefore be defined as a 'memory machine' able to collect, store, use and then transmit a wide variety of environmental information. The study of heredity is therefore the study of the different forms of information that can be transmitted across generations and affect parent-offspring resemblance. However, during the 20th century, due to the fantastic discovery of the DNA molecule and its incredible sequencic memory properties, we became blind to the existence of other types of transmission mechanisms. As a result, we have increasingly reduced heredity to its sequencic component, i.e. the sole transfer of the information encoded into the nucleotidic sequence of DNA, an attitude that I call sequencic. It is now time to re-open our views of inheritance to approach it in all its complexity. A first step to achieve this goal is to reflect about the gene concept.

Chapter 2
What is a gene?

It is not without irony that the two men who dealt the death-blow to the Christian Church's views on the creation of the universe and the assertion that the mineral and living worlds were created in their present states were a former Anglican priesthood student in Cambridge, England, called *Charles Robert Darwin*, and an Augustinian monk called *Johann Gregor Mendel*, abbot of the Abbey of St Thomas in Brno, in what is now the Czech Republic. These two men, in different ways, were very interested in sex, as we will see in this book, because how can we talk about heredity without talking about reproduction and sex? After all, we cannot talk about living beings without talking about reproduction and therefore heredity. For example, when, in the middle of the 19th century, *Mendel*, on the basis of a series of crosses of peas with different characteristics, established the first laws of heredity, he was looking for the rules of parent-offspring resemblance. Published in 1866, his laws, now known as Mendel's laws, initially went unnoticed, only to be rediscovered in the early 20th century. Today, Mendel's laws form the very basis of genetics, which was initially just the science of heredity in its broadest meaning.

Today, if you asked scientists their definition of a gene, you would probably obtain as many definitions as the number of answers you would get. Here, I only discuss two broad families of definitions of that concept, each corresponding to two periods that can be distinguished in the history of genetics. Until the discovery of the properties of the DNA molecule and the genetic code in the 1950s, i.e. for almost a century, although the actual carrier of hereditary information was unknown, the study of the mechanisms of heredity progressed enormously. The concepts of "gene" or "genetics" were invented and geneticists established all the major principles of genetics.

In 1953, the situation changed drastically. *James Watson* and *Francis Crick* on the one hand, and *Maurice Wilkins* and *Rosalind Elsie Franklin* on the other, established the polymeric structure of DNA. This molecule had been suspected of playing a central role in heredity for some time. But it was finally brought to the centre of biology by the realization that its nucleotidic chain structure had all the characteristics necessary to encode information. The subsequent discovery of the genetic code linking the sequence of nucleotides to the sequence of amino acids in proteins convinced even the most sceptical that the DNA molecule was a memory molecule *par excellence*, capable of encoding an enormous amount of information because of its enormous length.

This major and quite fascinating discovery gradually led to a total change in the very notion of a gene. This is what we will see in this chapter, as well as some of the major consequences that this history of two distinct periods still has on the way we think today.

The pre-DNA gene concept: an purely statistical concept

Throughout the first half of the 20th century, we did not know the exact nature of the carrier of genetic information. Even before 1930, however, it was known that genes were located in the nucleus of cells and more precisely in chromosomes, structures that are easy to stain with cytological dyes[29]. Speculation was rife, but before 1950 we had no proof. Therefore, unlike today, the gene concept was not based on any molecular support as it is today. The word gene referred to "*that which is passed on from parents to offspring, causing the latter to resemble the former* ". So it was a rather abstract and very open concept that I call the "pre-DNA" concept of the gene. At that time, approaches were mainly based on the statistical quantification of parent-offspring resemblance. This was typically the approach of Mendel and followers[30].

From Darwin to variance decomposition

Early in his book "*The origin of species*" published in 1859[31] Darwin wrote "*Any variation which is not inherited is unimportant for us*". Translated into positive terms, this sentence states that as far as the mechanism of evolution by natural selection is concerned, only the variation that is transmitted is important. He thus breaks variation down into two parts, the transmitted *versus* the non-transmitted components. With this one sentence, he expressed two of the conditions of natural selection, that there must be variation, and that this variation must be transmitted. That sentence actually defines heritability as we discussed in the previous chapter. It is the heredity of differences, the fact that big cats make big cats. Thus, *Darwin* understood that in order to study evolution one must account for the phenotypic variation among individuals of a given population, and focus

on the part of this variation that is transmitted to the descendants. In doing so, he laid the first foundations for a statistical approach to heredity.

Heritability: measuring parent-offspring resemblance

In practical terms, how could genetics be studied in those not-so-distant days? During this long period we were mainly interested in quantifying parent-offspring resemblance. It was known that this transmission was based on the transmission of 'something' found in the gametes of both sexes. In material terms, that was about all that was known. The approach to studying genetics was purely statistical, as *Mendel* himself had done. One sought to quantify statistically how variation is passed on to offspring, and the degree of parent-offspring resemblance for a given trait. If a level of similarity was found, it was concluded that this was the result of gene transmission (of course in this pre-DNA sense, which did not prejudge the medium of this information).

Figure 1: The classical method to quantify parent-offspring resemblance: regressing offspring trait on parent trait. In the theoretical case where children have exactly the same trait as their parents, the points would lie on the grey dotted line and the heritability would be 1. In real data, it can be seen that although there is some variation on the trait under study, there is a real tendency for offspring of parents with big traits to have big traits, and at least bigger than offspring of smaller parents. However, the slope of this relationship is significantly less than 1, indicating that offspring of parents with big traits tend to be smaller than their parents, while offspring of small trait parents tend to have bigger traits than their parents. This is a well-known phenomenon since *Darwin*'s cousin, *Francis Galton*, highlighted it in 1886 by establishing what is still known today as a regression[32]. It is the slope of the regression, here the slope of the solid black curve, which quantifies the degree of parent-offspring resemblance.

In practical terms, the best way to quantify this resemblance is to measure the trait under study (height, running speed, resistance to effort, skin colour, hair colour, emergence of a disease, etc.) in parents and offspring and to run a regression whose slope quantifies the degree of parent-offspring resemblance (**Figure 1: The classical method to quantify parent-offspring resemblance: regressing offspring trait on parent trait**. In the theoretical case where children have exactly the same trait as their parents, the points would lie on the grey dotted line and the heritability would be 1. In real data, it can be seen that although there is some variation on the trait under study, there is a real tendency for offspring of parents with big traits to have big traits, and at least bigger than offspring of smaller parents. However, the slope of this relationship is significantly less than 1, indicating that offspring of parents with big traits tend to be smaller than their parents, while offspring of small trait parents tend to have bigger traits than their parents. This is a well-known phenomenon since *Darwin*'s cousin, *Francis Galton*, highlighted it in 1886 by establishing what is still known today as a regression. It is the slope of the regression, here the slope of the solid black curve, which quantifies the degree of parent-offspring resemblance.

). This type of approach dominated genetics until the discovery of DNA.[33]

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The post-DNA gene concept

With the discovery of DNA and its fascinating sequencic properties, the very concept of genetics changed dramatically from being purely statistical and abstract to being molecular, material and sequencic. Accordingly, the tools for genetics have changed profoundly. From statistical, they became molecular. I remember that in the 1970s, some of my classmates at university told me that they had chosen to do molecular biology because they thought this emerging science was the future, which proved to be true.

Looking back, it is striking how quickly this transition occurred after the discovery of the DNA structure. For instance, as early as 1958 at a symposium of the Society for Experimental Biology, *Francis Crick* stated what was quickly coined as the "central dogma of molecular biology" formulated solely in terms of the sequence of macromolecules[34]. This fundamental revolution did not take five years to occur!

The first step was to study the amino acid sequence of proteins. Then, with the advent of DNA sequencing machines, and especially during the late 1990s, with the advent of high-throughput sequencing and associated bioinformatics, attention increasingly focused on sequencic. The first complete sequence of a human genome was published at the turn of the third millennium. The idea that ran through the media at the time, and unfortunately still does in some circles, was that having the human DNA sequence would allow us to cure genetic diseases. I guess at the beginning this was just a marketing argument to get the necessary funding, but the problem is that we often end up believing in our dreams[35]. While this was a necessary step towards gene therapy, it was probably only one step in a long journey of thousands of miles towards that goal.

Today, it is clear that high-throughput sequencing has led to a cascade of major discoveries, including those that are the subject of this book. But, while this massive increase in sequencing capacities should have marked the apogee of the sequencic model of life, this incredibly improved description of genetic variation has also led to the highlighting of the many limitations of this exclusively sequencic vision of life. It will be the objective of the rest of this book to convince you of the importance of all these discoveries. But before doing so, we must address a number of points.

How to reconcile these two visions of genetics?

You might say, "well, here we are with two very different understandings of genetics, but as they were introduced successively, this in itself is not a problem; today we know that genetic information is transmitted by the duplication of the DNA sequence during cell divisions and that the information it contains resides in the sequence of nucleotides in this molecule".

The two gene concepts coexist...

However, today the pre- and post-DNA gene concepts still coexist. As a matter of facts, the statistical conception is at the very heart of whole areas of biology such as quantitative genetics, evolutionary biology (see Glossary), functional biology (see Glossary) or epidemiology. Nowadays, these scientific domains still postulate that the statistically demonstrated parent-offspring resemblance can only result from the transmission of the DNA sequence. We will see many cases where this assumption is incorrect and how this can lead medical research into real dead ends.

The so-called 'genetic' diseases

In fact, there are three very different types of reasons for calling a disease as "genetic".

- First, in the vast majority of cases, a disease is said to be genetic only because it has been shown to be inherited. In other words, the offspring of people with a disease have an increased chance of developing the same disease. The argument is therefore purely statistical (pre-DNA). However, today the term genetic is always interpreted in its sequencic (post-DNA) sense. Although the transmission of the disease from parent to offspring is well documented and indisputable, the underlying mechanism remains totally unknown, as statistical arguments cannot be used to infer the transmission mechanisms. These could have a sequencic cause, but as we shall see, could just as easily have a non-sequencic cause. This is not just a semantic problem, because the purely sequencic interpretation of resemblance leads to ignoring any other form of inheritance, simply preventing the development of therapies to treat the concerned diseases. The medical impact of this confusion between the two meanings of the gene is therefore substantial, and it is at the origin of many delays in medical research.
- Secondly, in far less common cases, the label "genetic" results from the fact that the occurrence of the disease has been statistically associated with the existence of a mutation. However, as we shall see below, correlation does not imply causation, and again this can lead to a huge amount of time being wasted (I am talking decades) in pursuing false therapeutic avenues using only sequencic techniques, with huge human, financial and health costs.
- Finally, in very rare cases, there is a body of molecular evidence, involving very fine molecular techniques such as experimental silencing of specific genes (e.g. by interfering RNAs), that allows a causal link to be established between the dysfunction of a gene and the disease in question. It is only in these very rare cases that it can be said that there must be a sequencic component to the disease.

The first two types of argument represent the vast majority of cases. In those cases the term "genetic" is ambiguous, to say the least, and even dangerous, as it may prevent us from developing effective therapies. To avoid any ambiguity, it would be preferable to use an expression such as "transmitted or inherited disease.

... and we unconsciously move from one to the other

Despite this, very often the approach used to further investigate a transmitted trait or disease rests on the claim that it is 'genetic'. Why not, if one remained consistent with this pre-DNA definition, which would leave the door open to all types of inheritance mechanisms? But the problem starts when we realise that today, for all of us, when we say genetics, we think of DNA sequence, and mutation (i.e. change in the DNA sequence) and nothing else. And indeed, once the word genetics has been uttered, the natural next (and most often unique) step is to sequence the DNA or to use techniques to associate variation among individuals in the trait under study with a position in the DNA sequence.

Finding a gene that has the same mutation in a sample of patients that is absent in non-diseased people is a step forward, but by no means constitutes definitive proof that the observed mutation is the cause of the disease in question. It is only a co-occurrence of the disease and the mutation. B*ut co-occurrence does not mean a causal relationship*.

Co-occurrence does not mean causality

The question of the causality of observed phenomena is absolutely central to science[36]. Most of my readers know that a correlation (or co-occurrence) does not necessarily mean a causal relationship. Box 1 develop a funny example of the kind of mistake that can result from the interpretation of correlations in terms of causality.

Box 1: Storks don't bring babies.

Parents who do not want to explain to their offspring how babies are made, tell their children that babies are brought by storks. A clever child would be entitled to argue with his parents that hence the more stork nests there are in a place, the more babies there should be. That would make sense. Well, this is indeed the case across Europe where the number of stork nests per country correlates with the human birth rate in that country (**Figure 2**: Storks bring babies. A) Human birth rate is higher in European countries with more stork nests. Data from 17 European countries over the period from 1980 to 1990. B) Potential explanations for the existence of this correlation.

A)[37]. Clearly, from 1980 to 1990 in Europe, the countries with many stork nests had the highest birth rates (the probability of rejecting this correlation was only 8 in a thousand). Of course, it would not be serious to conclude from this that it is indeed the storks that bring the babies. Nevertheless, this is what we do whenever we cannot manipulate the system we are studying for ethical or technological reasons.

In this case a cleverer child could retort to his parents that introducing storks into the environment, or removing them from other places, should increase the local human birth rate in the first case and decrease it in the second case. He would then be doing a real experiment and that would be the only way to begin to demonstrate causality. Of course, such an experiment would fail, which would invalidate the original hypothesis.

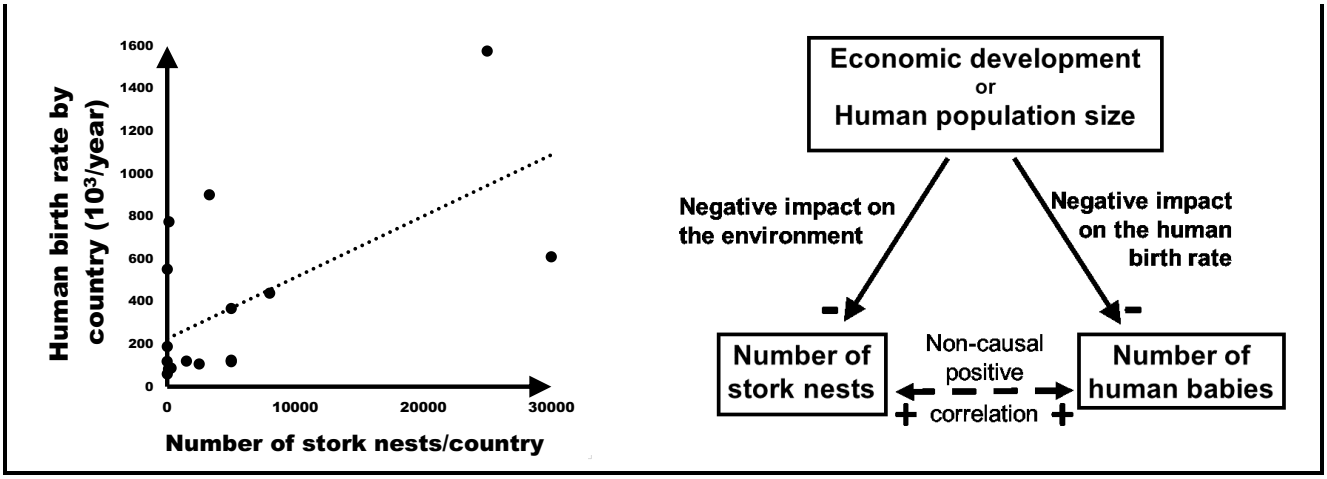
As is well known, such relationships can result from the effect of a third variable causally related to the two variables under study. Here, it could be the economic development or the human population size in the country (**Figure 2**: Storks bring babies. A) Human birth rate is higher in European countries with more stork nests. Data from 17 European countries over the period from 1980 to 1990. B) Potential explanations for the existence of this correlation.

B). Indeed, economic development or simply the country's human population size may negatively affect the quality of the environment and thus the number of stork nests. Similarly, we know that the human birth rate falls in parallel with the economic development, which creates a second negative relationship (**Figure 2**: Storks bring babies. A) Human birth rate is higher in European countries with more stork nests. Data from 17 European countries over the period from 1980 to 1990. B) Potential explanations for the existence of this correlation.

B). This would generate the observed positive statistical relationship between the number of stork nests and the human birth rate. In other words, there is no need for a direct causal relationship to generate a real relationship or covariation between two quantities.

Figure 2: Storks bring babies. A) Human birth rate is higher in European countries with more stork nests[38]. Data from 17 European countries over the period from 1980 to 1990. B) Potential explanations for the existence of this correlation.

A) B)



We cannot rely too much on correlations to study causality. We should be very wary of all the talks that tell us, for example, that it is good to have sex well into old age because couples who continue to have sex late in life are also the ones who survive the longest. This documented relationship between having sex and survival is most likely not causal. In this case a third causal variable could be people's health. Since healthy people continue to have sex in old age and survive longer, this generates a positive correlation between these two quantities, but it does not mean that it is having sex that increases survival. It may even be the other way around and that having sex in old age shortens the life span of people in poor health. Listen to the radio and you'll get plenty of examples of this kind of association being interpreted as cause and effect.

This is a very disturbing conclusion, because the objective of all science is to study causality. Should we then not be interested in correlations? Obviously not, and in certain fields such as medicine, it is the most common approach. But, we must avoid interpreting these relationships as causal *too quickly*. At some point, an experimental approach must be used to really address the question of causality. This is where animal experimentation comes in, to enable medical research that cures us every day to make progress in this major area of causality without which no therapy can be defined. Thus, there are cases where it is almost impossible to experiment to investigate directly the cause of the processes under study. This is particularly the case in medicine or conservation biology, where it is often impossible to conduct experiments for obvious ethical or technological reasons.

The "gene for something"

However, the inclination to use co-occurrence as evidence of causal links is not only present in medicine. It is also very common in genetics where co-occurrence is interpreted commonly as showing causality. For instance, some of our common expressions contribute to reinforcing this causal interpretation of co-occurrences. An example is the common use of the phrase "the gene for something". This is a convenient shortcut that nevertheless conveys a misconception that helps to convince us that everything is based on the DNA sequence and only on it. In the vast majority of cases, this expression is incorrect in more ways than one.

- First of all, the expression "the gene for..." implies a causal link between the presence of a particular sequencic variant and the study trait. However, as we have seen, in most cases, only the co-occurrence of the trait with a sequencic variant has been documented. As we have seen, correlation does not mean causation.
- For instance, two independent studies, each having observed a very strong association between a disease and a single mutation, can claim to have found *The* Gene' for the disease, even though each of these studies was talking about different areas of the genome, located on different chromosomes. This was the case, for instance, with bipolar affective disorder, which resulted in two publications in Nature in the same year, each claiming that the disease is so closely associated with one part of the genome that it could be said that "*These results confirm that a major psychiatric disorder can be caused by a single genetic defect*"[39], whereas the regions of the genome highlighted by these two studies were not even on the same chromosomes.
- This phrase seems to assert that a single gene is involved in the trait, whereas the vast majority of traits (if not all traits) are influenced by many genes, not just "the gene for...". The example of bipolar affective disorder above illustrates this fact.
- More generally, only very few of the 6,000 or so diseases classified as genetic have been shown to have a clear and well-established mutational cause. And yet, it is always these rare cases that are put forward as a typical example of the link between mutation and disease[40]. Obviously, these cases are far too rare to be set up as a general principle as we too often do when teaching genetics[41].

- Conversely, in the vast majority of cases, a given gene acts on a whole range of functions and affects a whole range of traits. This is called pleiotropy.
- Finally, genes are always named by the context in which they were discovered. This name is too reductive, because of pleiotropy, each gene could have different names, each related to one of the functions it affects. The name can even be misleading. For example, researchers working on wing development in fruit flies identified a gene whose mutation led to the disappearance of wings. They therefore called it *Wingless*. In parallel, teams working on mice identified a gene that favours the emergence of breast cancer. They called it *Int-1* and considered it to be a breast cancer gene. However, when these two genes were sequenced, they were found to have almost the same sequence and to be the same gene inherited from a distant common ancestor. It is neither a wing gene nor a cancer gene. It is involved in a cell-to-cell communication function and its effect depends on the cell and the organism in which it is expressed. The new name for this gene is *Wnt*, which is a contraction of its two former name[42].

Of course, we have to give a name to all the concepts we invent. The same is true for genes. But we have to handle these names very carefully because they are reductive and therefore incorrect. In very general terms, we must be very careful not to believe in the names of genes. And the best way to avoid this trap is to never again use the expression "the gene for..."[43].

There is no equivalence between transmission and DNA sequence

Thus, despite their great difference, the two conceptions of genetics, statistical *versus* molecular, persist and intermingle all the time, which leads us to often unconsciously switch from one to the other within the same project, the same paragraph, or even the same sentence, thus maintaining a particularly deleterious ambiguity.

In fact, once a trait or disease has been shown to be statistically transmitted, the switch to sequencing implicitly means a major reduction in our field of thoughts. It is a major quantum leap from the statistical measurement of a population-wide transmission to the study of the DNA sequence, and most of the time, to nothing more than that. The problem is not in trying to see what part of the DNA sequence might be involved, because that can provide useful information, but the problem is that we get stuck with that single approach, which implicitly assumes that because it's transmitted, it must necessarily be the result of variation in the DNA sequence. This prevents us from looking elsewhere and *de facto* eliminates the possibility of transmission of any other nature than that encoded into the DNA sequence. And since we only find what we look for, it prevents us from discovering and studying any other form of inheritance that is not linked to the DNA sequence.

The history of biology is full of examples where the causal equivalence 'sequencic ⇔ transmission' proved to be incorrect[44]. Yet this equivalence is implicitly made every day by both the media and scientists. Originally, it was the scientists who claimed that this or that disease or trait was genetic, on the basis of statistical arguments that cast doubt on the fact that this implied a sequencic coding. But how do we ultimately define a gene?

What is a gene?

The solution to reconciling these two conceptions of the gene is to give them different names, each of which is always used in the same and unique sense. This is my aim in the end of this chapter. But before I do, I will use a metaphor to make a point about the deeper nature of the concept of a gene.

Spielberg's latest film

If I asked you what Stephen Spielberg's latest film was and you said "This is it" while handing me a compact disc, I would be entitled to be dissatisfied with your answer[45]. After all, I asked what this film is about, who the actors are, how the photography is, the music, the rhythm of the film, the quality of the acting, etc., all information that would have allowed me to make an idea about this film. Instead, you give me a compact disc, which can be of various formats, CD, Blue Ray, or other. You could also have given me a magnetic cassette (although they don't really exist anymore) or a USB key with a file that could have had different formats, or even the reels of the film itself. The weight of these various media varies from several tens of kilos to a few grams, but all contain the entire film itself.

Avatar vs information

When I asked you about Spielberg's latest film, I was not interested in the material support, but something abstract, which is the story of that film. We must therefore distinguish between the information that this film conveys and its avatar[46], that is to say the material form taken by this information.

The same applies to the concept of a gene. If you were to ask random people what is a gene, most often you would say something like "It's a piece of DNA", possibly followed by "that codes for a protein". The first part of this answer is talking about the avatar. The second part is also about another form of avatar. More rarely

people would add "that performs a certain function (or functions) in the functioning of an organism". Here we would start to leave the material notion and go to a more abstract dimension, that of its "function".

A gene is information

What matters in a gene is not so much the avatar as the information carried by the avatar[47]. What is selected is not the piece of DNA, but the information that this sequence carries. It is this information that is transmitted, not the DNA molecule itself. This information is conveyed by a copy of the DNA, allowing it to participate in the reconstruction of a new individual that will resemble its parents because it has received the same information. Similarly, if today we believe that there are other forms of life on other planets, we do not know what they would look like. What we can say, however, is that these life forms would have to rely on the transmission of information between generations. However, it may well be that the information avatar enabling these other life forms is of a very different nature to that which exists on earth. But regardless of this, we could still use the word gene to describe this information, even though it is carried by another avatar, because it would serve the same function.

A clear terminology of gene concepts

To reconcile these two visions of genetics, it is necessary to give each of them a precise name and to stick to them.

Post-DNA or sequencic definition: the information encoded into the DNA nucleotidic sequence

Since there is no point in fighting a predominant use of a concept by trying to restore it to its long-forgotten original meaning, I will restrict the concept of gene to its reduced, sequencic understanding.

According to this view, when we say gene or genetics, we clearly refer to the information engraved into the nucleotidic sequence of the DNA molecule[48]. This sequencic view then allows one to state that any information transmitted across generations through a channel other than the DNA nucleotidic sequence is of *non-genetic* nature. Indeed that this definition of genetics is highly reductive, but it has the merit of being very clear and of being limited to a single type of biological information, which we know has relatively homogeneous properties of transmission and stability over the generations (mutation rate, etc.).

Inclusive heritability = the pre-DNA definition of the gene: parent-offspring resemblance

To invoke the statistical pre-DNA concept of genetics is to address the level of parent-offspring resemblance, the measure of which is heritability, discussed earlier in this chapter. But here again, ambiguities about the gene concept have gradually altered the definition of heritability, that nowadays is taken to mean the part of the variation that is transmitted *genetically* (implicitly, in this case sequencically). To get around this problem, I proposed 10 years ago[49] a broader definition of heritability, which I called inclusive heritability[50], and which in fact returns to the original meaning of heritability, i.e. the heredity of differences regardless of the mechanism of resemblance. *Inclusive heritability* is the part of variation that is transmitted, regardless of the transmission mechanism involved (be it genetic or not).

According to this terminology, when the offspring of sick people are more likely to inherit the same disease, we should no longer speak of a genetic disease, but rather of an "inclusively heritable disease" or a "transmitted disease" or an "inherited disease". There would then be no more ambiguity about the meaning of the term.

Even eminent scientists persist in maintaining ambiguity

When I talk about non-genetic inheritance, some of my colleagues, even those very close to me, say to me, "*in fine*, what you call non-genetic inheritance is part of genetics". I tell them that according to the pre-DNA conception of genetics they are right, because this conception is inclusive in the sense that it includes all forms of transmission that lead offspring to resemble their parents. The problem is that after making this point to me, these same eminent colleagues continue to work by considering only variation in the DNA sequence. In other words, we do not hesitate to maintain the ambiguity between the two understandings of the concept of genetics as an alibi for continuing to work as before, ignoring the fact that a significant part of the transmitted variation we are working on is not encoded into the DNA sequence.

I particularly remember one lecturer who had determined the genetic region responsible for the variation in the trait under study, but despite much effort had not been able to find the underlying genetic (sequencic) variation. I then pointed out that, perhaps, variation was of another form, such as epigenetics, but when asked how to solve this conundrum, I was given evidence that I had not been heard. Instead of saying, "I know the region involved and probably the variation in question is of a different nature than sequencic", the answer was "brutal sequencing". In other words, despite my suggestion, the idea that the variation might be something

other than sequencic variation did not even cross the mind of this renowned person. This shows how stuck we all are in the dogmas of sequencic.

Conclusion

From now on in this book, I will apply the definitions proposed above. When I say genetics, I mean sequencic, and by gene I mean information encoded into the DNA sequence. This is the common meaning of the term today. When I want to talk about the pre-DNA understanding, I will use the equivalent terms 'inclusive heritability', or 'parent-offspring resemblance', or 'statistical gene', or 'inheritance', or 'genetic and non-genetic', or simply 'pre-DNA gene', depending on what I want to focus on.

Chapter 3
Heredity according to the Modern Synthesis of Evolution

Can you imagine that for most of the first half of the 20th century, there were two independent disciplines that largely ignored each other, one dealing with natural selection and the other with genetics? Today this seems rather incongruous, but in fact these two disciplines did not really talk to each other for decades! Darwinians studied the mechanism of natural selection as the engine of evolution, while geneticists and biometricians studied Mendel's laws (the laws of transmission). It was population genetics that later showed the compatibility between Mendel and Darwin. This was the driving force behind the emergence during the 1940s-1960s of a synthesis between these two disciplines called the *Modern Synthesis of Evolution*[51], or the Neo-Darwinian Synthesis, or simply Neo-Darwinism[52] to emphasize the fact that it is an extension of Charles Darwin's original theory, who at his time ignored the mechanisms of heredity.

Today, the Modern Synthesis of Evolution is the mainstream view of evolution in the scientific community. The aim of this chapter is to give some important elements of this synthesis. This will allow us both to better understand the foundations of the ongoing scientific mini-revolution and to ask how these profound changes have the potential to change our view of life and its evolution. In particular, it will then allow me to better define what I call the *Inclusive Evolutionary Synthesis*[53] as we will develop it in the third part of this book.

A reminder

We saw in Erreur ! Source du renvoi introuvable. that the process of natural selection is triggered when there is variation in a given trait in a population, and when different values of the trait lead to individuals having more or fewer offspring. These two conditions will lead to an evolution of the trait towards the value(s) associated with a higher fitness if a third condition is met, namely if the trait is inclusively heritable. It appears, therefore, that the question of the sources of variation among individuals in a population is central to understand evolution.

Nature and age of phenotypic variation in a population

In the framework of the Modern Synthesis, the variation between individuals (called V_P for phenotypic variance) has two major sources.

- Individuals first differ in their genes. The age of this genetic variation is represented by the light blue area in Erreur ! Source du renvoi introuvable. and is measured as genetic variance (called V_G).
- Individuals also differ in their life experience. For example, some have developed in a poor food environment, others in richer environments. The former will be smaller on average than the latter. The age of environmental effects is represented in Erreur ! Source du renvoi introuvable. by the green area and participates in the so-called environmental variance (called V_E). These effects are expressed during development, i.e. after fertilisation. Nonetheless, in Erreur ! Source du renvoi introuvable. this green zone extends slightly upstream of fertilisation to take into account parental and grandparental effects that contribute marginally to modifying the phenotype of individuals.

This view of the sources of phenotypic variation leads to a very simple equation:

$$V_P = V_G + V_E$$

The effects of genotype environment interaction (denoted G*E) or their covariation (denoted cov(GE)) are often added to the right-hand side of this equation, but these are secondary points for my purposes here. This equation states that what matters for heredity and therefore for evolution is V_G, our goal being to estimate it. However, this equation can be read in two ways:

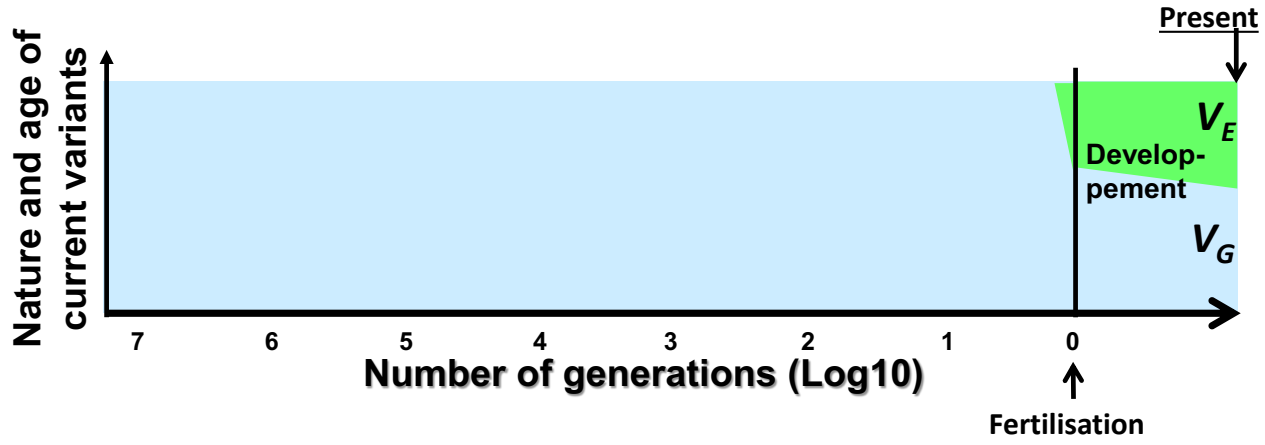
- In the original version of this equation, the word genetic had a pre-DNA meaning and included all the transmitted variation that produces parent-offspring resemblance. So this equation says that what matters for evolution is the part of the variation that is passed on to the offspring[54], which according to today's knowledge

certainly includes the transmission of the DNA sequence, but much more. It is only in this sense that this equation is valid.

- However, today we read this equation with the post-DNA meaning of genetics, which makes it invalid. Thus, the original meaning of this equation has gradually changed within the Modern Synthesis. Understood in this way, it amounts to stating that the only important term for natural selection is the variation in the DNA sequence and nothing else. In order to study it, it is therefore necessary to get rid of all environmental effects in order to try to purify sequencic variation as much as possible. This reading profoundly distorts the meaning of this equation because all variation that is non-sequencic but nevertheless transmitted is implicitly incorporated into the environmental variance in order to be eliminated, whereas the fact that it is transmitted implies that it is *de facto* part of inheritance and therefore of the evolutionary process.

In fact, with this second meaning, this equation is diverted from its initial meaning and claims that the only variation that matters for evolution is sequencic variation, the rest being considered as noise to be discarded in order to concentrate on sequencic effects. We will come back to this major point in the third part of this book.

Figure 3: Nature and age of the sources of extant variation among individuals according to the Modern Synthesis[55]. Time runs from left to right along the horizontal axis in base ten logarithm of the number of generations before the moment of fertilisation that gave rise to the study individuals. The value 1 means 10 generations ago, 2, 100 generations ago, and 3, 1000 generations ago and so on, with 7 meaning 10 million generations ago. So it's a broad time scale. The vertical axis represents the proportion of genetic (light blue) versus environmental (green) variation in the current population that existed at any time in the past and has therefore persisted until now. The colour represents the type of variants that have survived to the present day, out of all those that already existed in the population at that point in the past. The light blue area represents the genetic (sequencic) variants that are reflected in the current population by the genetic variance (V_G). Similarly, the green area represents the environmental effects that affect the phenotype of individuals in the current population. These effects are estimated by the environmental variance (V_E). According to the Modern Synthesis, none of the environmental effects would pass the generation barrier, with the small exception of parental and grandparental effects, which justify the fact that the green area extends slightly into the past to the left of the date of fertilisation. Thus, still according to the Modern Synthesis, beyond two generations in the past, 100% of the current variants inherited from the past are of genetic nature (for these periods, the entire area is therefore in light blue); no variant of environmental origin can persist for more than two generations, and the cases where variants resulting from environmental effects persist over one or sometimes two generations remain marginal.



The diagram of information flows across generations in the Modern Synthesis

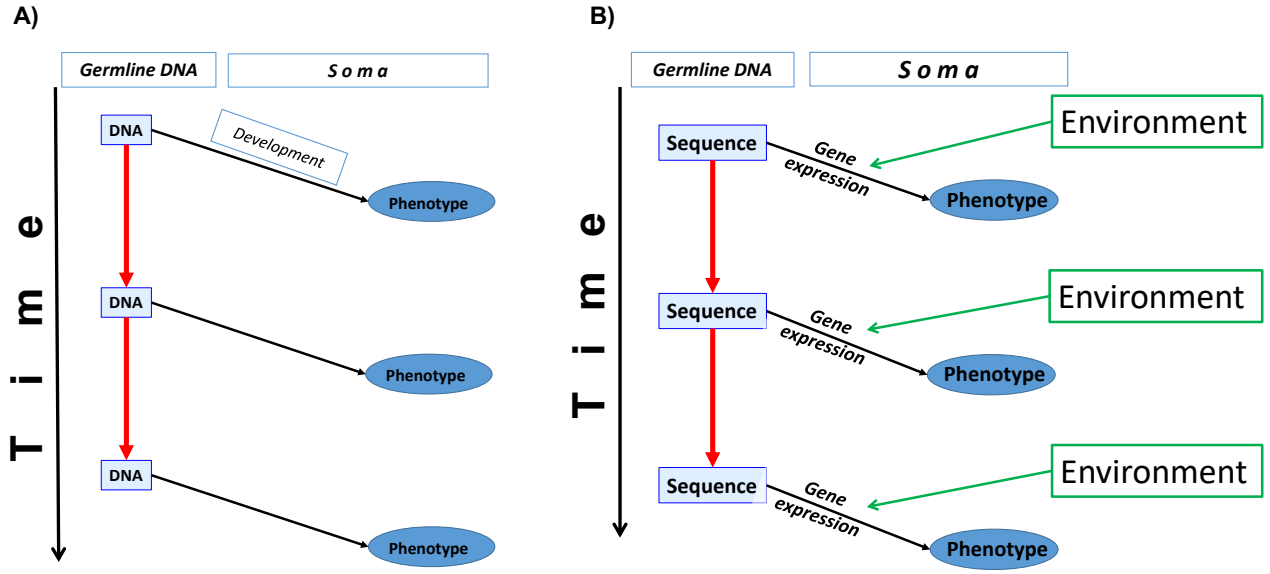
Now that we have a better understanding of the sources of phenotypic variation, we can address the question of how this variation is transmitted across generations, i.e. how inheritance works. The best way to do this is to construct a diagram of information flows across generations based on the distinction made in 1889 by *August Weismann* between what he called the "*germ plasm*", a concept close to that of *genotype* (see Glossary), and what he called the "*soma plasm*", which is close to the concept of phenotype[56]. In fact, *August Weismann's* main message was that in order to understand inheritance and evolution, it is also necessary to study development, an idea to which we will return later, because this message has in fact been largely overlooked, although it was central.

To illustrate his point, Weismann produced a diagram which was later simplified in two stages by *Edmund B. Wilson* in 1896 and by *John Maynard Smith* in 1965[57] (Erreur ! Source du renvoi introuvable.**A**). It should be noted that *Maynard Smith* replaced *Wilson*'s G (for 'Germ cell') with the word DNA. This was a clear statement of his support for the sequencic view of heredity, which was quite understandable at the time. To take into account Weismann's argument about the role of development, the Modern Synthesis added two fundamental elements (Erreur ! Source du renvoi introuvable.**B**), the fact that the environment plays a major

role in the development of the phenotype, and that this effect of the environment goes through a fine regulation of gene expression, a regulation that comes under what we call epigenetics today. Erreur ! Source du renvoi introuvable.**B** therefore, represents the canonical view of inheritance according to the Modern Synthesis, which integrates the important role of the environment in development**.**

The important point is that, according to this view, the only information flow across generations concerns the transmission of the DNA sequence across generations (there is only one red arrow in Erreur ! Source du renvoi introuvable.).

Figure 4: Diagram of information flows across generations. Each of these two diagrams represents three generations. A) The diagram by *Maynard-Smith* slightly completed and tilted to make vertical transmission vertical. B) A revision of this diagram with the addition of the two fundamental elements brought by the Modern Synthesis[58]. According to this view of inheritance, in the long term there is only one pathway of information transfer across generations, the one involving the transmission of the DNA sequence (represented here by the red arrows between successive generations).



The four main approaches to study evolution

The Modern Synthesis rests on several pillars, not the least of which is the one proposed in 1963 by *Niko Tinbergen* in a landmark article[59]. The general idea of this article is to show that when we ask ourselves the question of why we observe such and such a trait, there are four types of complementary approaches to answer it. I will illustrate these four types of approach by taking the case of male passerine song in spring.

The dawn chorus of passerines

In the late 1990s and early 2000s, one of my PhD students, *Amélie Dreiss*, studied the dawn chorus of forest passerines. She studied the blue tits (*Cyanistes caeruleus*) in the Orient Forest, which by a funny coincidence is located in the Aube department (*aube* in French meaning dawn). I often accompanied her in the field with a whole crew of students, forcing me to get up around 4 a.m. to get to the forest before dawn. Have you ever walked in the forest in early spring before daybreak? If so, you must have noticed that shortly before dawn all the bird species sing loudly and abundantly for about twenty minutes, then fall silent. This is the dawn chorus. As the time of daybreak advances rapidly during this season, the time of the start of the chorus also advances, so that it always occurs just before daybreak. This remarkable phenomenon naturally leads to the question of why the birds sing in spring.

What *Tinbergen* formalised that in biology, any "Why?" question can be answered in four broad ways. These are often referred to as *Tinbergen*'s four questions (or better answers).

A response in terms of immediate or proximate mechanisms

A first type of approach is to study the within organism processes that produce the trait in question. These are called *proximate mechanisms*. For example, we might say that birds are sensitive to the photoperiod, which, through a combination of hormones and neuro-hormones, stimulates males, leading them to sing when the days get longer. We could also say that song is a variation in air pressure that propagates in space like waves when a pebble is thrown into the water, and that our ear detects these vibrations that we call song. These vibrations are produced by an organ called the syrinx. Furthermore, we now know that birds probably sing

very early in the morning because the physical conditions of the early morning air are particularly favourable to sound propagation[60].

A response in terms of development

A second approach studies the developmental processes that lead males to produce the songs typical of their species. In this case, we can study the learning of song by young males of many passerines when they are in the nest and hear their father and neighbours singing. Again, this approach can be seen as focusing on the proximate factors that generate song.

A response in terms of intergenerational (or ultimate, or distal) mechanisms

A third approach concerns an even longer time scale by studying the advantages provided by the trait in terms of fitness, i.e. in terms of the ability to have offspring. For example, for male singing, the benefit in terms of fitness is that a male that would not sing would not attract a female. He would not reproduce and his lineage would become extinct. We are therefore interested in an intergenerational process, and we talk about *ultimate factors*.

A response on a macro-evolutionary scale

Although they take place on increasing time scales ranging from seconds to several generations, the three previous approaches remain on short timescales relative to that of evolution. They are said to concern micro evolutionary processes. The last approach unfolds over hundreds or more generations, in what is called a macro evolutionary approach. In the case of passerine song, for example, we could study how present-day singers have inherited the type of song of their species from a distant ancestor living millions of years ago. For this purpose, there is a whole range of methods for reconstructing ancestral traits even in the absence of fossils.

Tinbergen's last comment

At the end of his paper, *Tinbergen* emphasised that while each of these approaches is valid in its own right, all are necessary to understand the evolution of a trait. Only the synthesis of these four approaches can really allow us to hope to understand evolution. Some colleagues like *Michel Vancassel*, who worked at the University of Rennes in Brittany, regularly pointed at that important element of that paper. *Ernst Mayr* also proposed a similar idea in his important article on the question of causality in biology[61].

This integrative vision of the study of evolution was absolutely remarkable in 1964 and remains so today because scientists often confound these approaches, or consider that only one type of approach is serious, the rest not really being science. So more than 55 years after the publication of this important article we still have a long way to go to really integrate all these approaches. This book belongs to this integrative vision because it proposes a new evolutionary synthesis combining infra-individual approaches (Tinbergen's answers 1 and 2, which Mayr called functional biology[62]) with supra-individual approaches (Tinbergen's answers 3 and 4, which Mayr called evolutionary biology). In this respect, Mayr and Tinbergen were in some ways very much ahead of their time, since their aim was clearly to promote the emergence of an approach that would integrate the two major disciplines of biology, namely functional and evolutionary biology[63]. We shall see in the third part that this is exactly the aim of the Inclusive Evolutionary Synthesis, the definition of which is the ultimate purpose of this book.

My investment in Tinbergen's fifth response

I often group these different approaches into two sets, infra-individual (answers 1 and 2) *versus* supra-individual (answers 3 and 4) because it is often between these two groups of approaches that communication between scientists is difficult. My conviction of the need to adopt such an integrative approach led me to write with 4 other colleagues and then, once funded, to co-direct for 10 years with my colleague *Dominique Roby* a *Laboratoire d'excellence* (LabEx) entitled TULIP, whose central objective is to lead the communities in Toulouse that adopt infra-individual approaches (originally mainly on plants) to work with the community using supra-individual approaches[64]. TULIP is thus in line with Tinbergen's fifth answer and Mayr's vision.

Some major principles of the Modern Synthesis

Historically, the Modern Synthesis of Evolution was the product of a collective effort. There is neither a precise date nor a founding book sanctioning its birth. The Modern Synthesis marked the realisation that there was total compatibility between the Mendelian conception of heredity and the Darwinian conception of evolution by natural selection. This collective awareness, which allowed the synergy of these two vast disciplines, came about thanks to the work of a large number of people such as *Ronald Aylmer Fisher* (1890-1962), *John Burdon Sanderson Haldane* (1892-1964) and *Sewall Wright* (1889-1988), among many others, and later people like *Ernst Mayr* (1904-2005), *George Ledyard Stebbins* (1906-2000) and *George Gaylord Simpson* (1902-1984)

who helped to illustrate the explanatory power of this 'synthesis' in zoology, botany and palaeontology respectively, as well as Julian Huxley (1887-1975) and Theodosius Bobzhansky (1900-1975) as well as François Jacob (1920-3013) and Jacques Monod (1910-1976).

Today, in addition to making an equivalence between the information transmitted across generations and that encoded in the DNA sequence, the Modern Synthesis is based on a series of principles that, as is often the case with principles, are not open to discussion. However, numerous discoveries suggest that it is necessary to nuance some of them. To finish the first part summarising the main lines of the Modern Synthesis, I will now describe some of its major principles.

Unit of selection and selfishness

In 1976 *Richard Dawkins* published a landmark book entitled "The selfish gene". Central to the book was the concept of a *replicator* (see Glossary), an entity that can make copies of itself[65]. The common assertion that genes are such replicators is in fact a convenient shortcut because genes are not capable, on their own, of making copies of themselves. The DNA molecule on its own is unable to replicate itself and it is the complex cellular machinery in its environment that produces such copies[66]. These copies include all the changes that have happened to them since they were created, allowing them to evolve.

Dawkins' book has been translated into many languages and has been reprinted several times, indicating that it marked an important step in the history of the Modern Synthesis that now constitutes the mainstream conception of evolution. I will return to this work, for which the present book can be seen as a kind of update, but at this point it is important to emphasise that one of Dawkins' main points was to define what entity is actually selected, the so-called unit of selection. To illuminate this important idea, Dawkins used the term *'selfish'* (see Glossary), a term for which he was often criticized[67], while the real contribution of his book was to the unit of selection. His message was that although expressed in phenotypes, selection *in fine* produces a *response to selection in terms of changes in gene frequency in the population* and that, as such, a gene can be defined as a unit of selection.

The Weismann rule (or barrier)

Weismann's separation of the germline from the rest of the body, often called the *soma* (see Glossary) has led to the proposal that there is a barrier, classically called the *'Weismann barrier'*, protecting the germline cells (those that will produce gametes) from environmental effects. In other words, no matter how strong and intense the environmental changes are, the germline should not be affected, so that the information passed on to the next generation is completely unaffected by any environmental effects during the lifetime of individuals.

There is an indisputable logic to this crucial idea. Without such a barrier, all the mutations and environmental effects accumulated during life that ultimately lead to death would be passed on to the next generation, which would quickly lead to the extinction of life. However, we will see that we need to revisit this so-called concept of Weismann barrier[68], by nuancing or adapting it somewhat.

Mutations are random in relation to the environment

A basic tenet of Neo-Darwinism is that the environment can never drive mutations in the direction of improving *adaptation* (see Glossary) to the environment. This is absolutely true, and to my knowledge there is no evidence to challenge it.

However, this statement is often rephrased by saying that mutations occur randomly in the genome. The issue of randomness in biology is very complex, and some of the interpretations of this statement may be incorrect. Similarly, mathematical models used to understand the functioning of living things often assume that mutations occur completely at random because this greatly simplifies the writing of equations. I suspect that it is this mathematical constraint that has led to the establishment of randomness as a general rule. However, it must be the properties of living things that govern the mathematical formalism and not the other way round. In other words, if biology questions part of a formalism, then it is up to mathematics to adapt.

Let us return to the importance of randomness in biology. In a very general way, I must repeat here that *there is no need for any external force (call it what you will) to influence mutations, and thus to direct evolution in an a priori direction*. I want to say this firmly here, because people sometimes associate this kind of idea with my views. Evolution can be explained entirely by the properties of living organisms, without the need for external interventions of any kind. And this is partly due to the fact that randomness is sufficient to explain the genesis of variation and therefore the functioning of living things.

However, it is not necessary to invoke randomness everywhere, even where observations show that randomness is probably partly influenced by the environment. Even if it is clear that the environment can in no way direct mutations in the direction of an improvement in the individual's adaptation to the current environment, it is possible that the environment can favour the appearance of mutations in the areas of the genome that are precisely involved in *accommodation* (see Glossary) to the specific environmental change (we

will return to this question in Chapter 10 and Chapter 15). So the mutations would occur, indeed independently of the environment, but the environment would favour their emergence in target regions of the genome that are non-neutral in relation to the response to the environment. If this were the case, then it would significantly restrict the type of randomness involved. This is a delicate issue that is related to the following principle.

a sup
a sup
a mis
a mis

Finalism

I have often asked students at all levels whether they have been confronted with the recurrent claim that *finalism* (see Glossary) is to be banned. I can't remember a single time when a whole class told me that they had never heard this kind of statement. And indeed, even among scientists, people are regularly accused of finalism when they say, for example, that "male passerines sing in spring *to* attract females". In fact, the use of the word 'to' in this sentence is a slightly abusive shortcut, and it would be enough to say "passerine males sing in spring, *which has the effect* of attracting females" for no one to react.

However, this second formulation is itself highly finalistic because it presents a direct causal relationship between singing and being able to reproduce. This clearly shows that there is a finality in biology, and this finality is called fitness. It is natural selection that directs the selection for those individuals that, given their characteristics, do best in terms of offspring production. There is therefore "*de facto finality*" (see Glossary), which is to transmit to future generations. To deny this would be pointless. If you are reading this paragraph, it is because since the first appearance of self-replicating entities some 3.5 billion years ago, the chain of generations has never broken to eventually lead to you. At every point in this gigantic history there have been organisms from which you are descendant. One interruption and you would not be here. This is true for all currently living organisms.

The existence of *'de facto* finality' should therefore not be denied. And that is the nuance. When people reject finalism, they are not rejecting *de facto* finality, but rather any *teleological finality* (see Glossary), i.e. the idea that there is an external force that directs the system. Again, there is no need to invoke such an external force. The properties of living beings are sufficient to explain evolutionary dynamics.

My annual activity reports at the CNRS illustrate the power of *de facto* finality to generate a history that seems written in advance when recapitulated *afterwards*. At one point in my career I transformed my annual report into a narrative of my research history. In doing so, I realised that this gave the impression that I had followed a logic from the moment I joined the CNRS that led me straight to the writing of this book. Nothing could be further from the truth. However, once I had retraced my steps (which is what any phylogenetic tree does), my story seemed to follow such a pre-organised logic, as if an external force (in a sort of teleological finality) had directed me towards this book. I thus added a remark at the beginning of my report specifying that this was a false impression. There was much more randomness in the course of my career than my report suggested. However, there was a real finality to the whole thing: I had to publish. This had been the selection pressure exerted on me by my institution. This shows to what extent a specific selection pressure (to publish) can generate, in interaction with the environment, a story that *a posteriori* seems to have been directed from outside towards a given goal. This is pure illusion; the importance of publication (the equivalent of fitness for living organisms) constituted a selection pressure that led to the unfolding of that specific story.

So we should not reject all forms of finality in biology, only teleological finality should be rejected. Life is directed by the *de facto* finality of producing a few more offspring than other population members. Otherwise, in the long run, the lineage runs the risk of becoming extinct, and all living beings around us are present because their lineage has never stopped since the first living organisms appeared. This is an indisputable fact.

Acquired traits cannot be transmitted

An impossibility...

One of the major tenets of the Modern Synthesis is that there are no Lamarckian processes, often described by the phrase "heredity of acquired traits", which is actually quite far from what Lamarck said. However, this principle is clearly challenged by numerous recent and well-documented discoveries of molecular mechanisms showing the undeniable existence of some forms of heredity of traits that arose in response to the environment.

When, at the beginning of the 19th century, *Jean-Baptiste Lamarck* formalised the idea that species change over time (i.e. evolve), he took humanity a great step forward in its knowledge of living entities. It was a revolutionary idea in his time, as it was still in Darwin's time six decades later. However, in both their times, nothing was known about inheritance mechanisms, nor about its rules, nor about the way in which hereditary information is encoded. Lamarck proposed the "rule of use and disuse" as the general engine of evolution. For his part, 50 years later Darwin proposed natural selection as a general mechanism of evolution. But several points need to be made here.

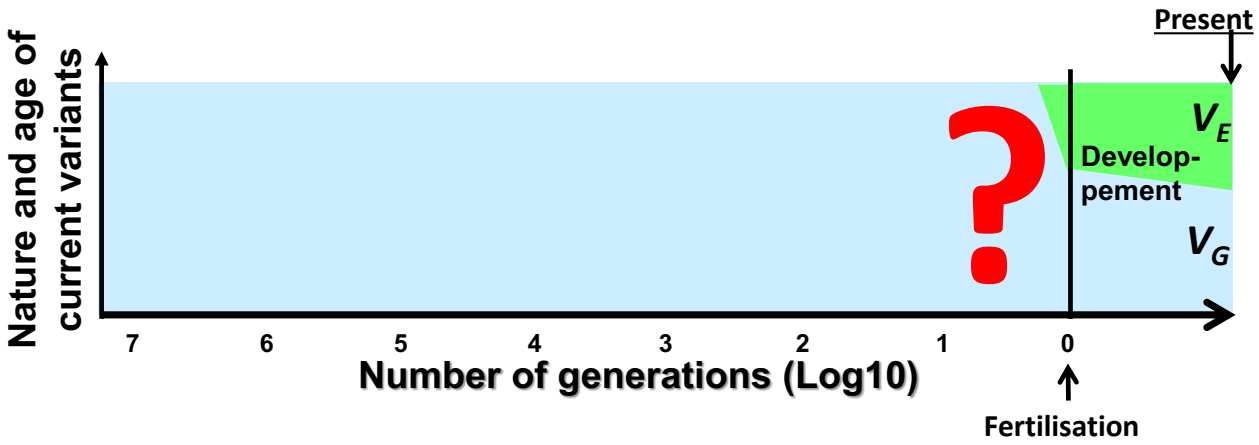
First of all, Darwin built on Lamarck ideas. In other words, Darwin would probably not have existed without Lamarck. To oppose them would be equivalent to opposing ideas published in 2020 to those from 1960, which would not make any sense. Moreover, contrary to a generally accepted idea, these two mechanisms are not unfolding at the same organisational and temporal levels and are therefore not at all incompatible. This is all the more true since Darwin had a Lamarckian vision of inheritance, which did not prevent him from imagining and proposing the very powerful mechanism of natural selection. There is therefore no incompatibility between these two processes because, as we will see in the third part of this book, these two processes do not function at the same timescales.

...that is nevertheless supported by simple logical reasoning

In looking at Erreur ! Source du renvoi introuvable., one can be struck by an important enigma, as according to this diagram, only genetic information, itself inherited from very ancient ancestors, is passed on to offspring (with the marginal exception of parental effects). This is surprising, because it is very likely that the environment of recent ancestors would be a much better predictor of the environment in which descendants will live than that in which lived ancestors of thousands or even millions of generations ago. By virtue of its very high transmission fidelity, genetic information is in effect specialised in the transmission of information inherited from very distant ancestors. It therefore seems that in a population of this type, any organism that would acquire the ability to transmit information about the current environment would have a major selective advantage because its descendants would be moulded, as it were, to the environmental conditions in which they will have to develop, survive and reproduce. In a way, their descendants would be informed, or warned. Thus, the absence of this kind of transmission constitutes a real enigma. This is why I placed a big question mark at this point in Erreur ! Source du renvoi introuvable.

This reasoning leads us to speculate that there must be in nature mechanisms of transmission of newly acquired abilities in the lineage.

Figure 5: The enigma of non-transmission of acquired traits. Why is it that recent ancestors do not transfer information about the state of the environment, while the environment they live in is undoubtedly a much better predictor of the environment their descendants will face than that of ancestors who lived millions of generations ago? See the legend to Figure 3 for axes definition.



Summary

Today the mainstream view of evolution is called the Modern Synthesis of Evolution. According to this view, inheritance essentially, if not exclusively, involves the transmission of the information encoded into the DNA sequence. This is in fact a relatively reductive view of evolution that is based on a number of principles, some of which are currently being challenged by recent results. This situation calls for a new synthesis that would integrate these new discoveries.

The aim of the second part that we are now going to address is to illustrate many well-documented facts, especially concerning the underlying molecular mechanisms of inheritance, that show the existence of unsuspected mechanisms of inheritance. As science is the domain of facts, the existence of new facts must lead us to change our conception to one that integrates all the documented facts. It will be the aim of the third part of this book to propose such a new synthesis.

Part Two

Non-genetic inheritance

I must confess that in the late 1990s, if you had asked me whether the only information really transmitted from parents to offspring is the one that is encoded into the DNA sequence, I would most likely have answered positively. In so doing, I would have conformed to the mainstream Neo-Darwinism view that I just briefly described above. This is what I was taught at university in the 1970s and what I still continued to teach at that time. However, this Neo-Darwinian vision, which was then at a sort of apex, was soon to show many limitations, mainly as a result of the discoveries that accumulated at a steady pace from the beginning of the third millennium. The irony of this story is that it was the advent of high-throughput sequencing, a revolutionary technology destined to establish the Modern Synthesis once and for all, which, by enabling us to change the scale of our approaches, exposed the limitations of this dominant vision.

My goal in this second part is to illustrate the vast range of the discoveries revealed by the widespread use of these new technologies. It is difficult to describe the perplexity, and then the intellectual excitement, that these discoveries produced in me as I discovered their extent.

Chapter 4
The case of the missing heritability

Imagine the enthusiasm of evolutionists when we finally had access to the entire human genetic sequence![69] Everything was there, we thought, written in this fantastic sequence that would allow us to understand and alleviate our diseases! There was reason to rejoice. Admittedly, this first sequencing had cost around 3 billion dollars, but it was worth it, because we were finally going to be able to associate genetic variation with phenotypic variation! This had been the hidden dream of every biologist for a long time. Moreover, this first sequencing led to the emergence of high-throughput sequencing and a whole series of other molecular methods enabling very fine descriptions of genetic variation among individuals within a population. After all, the uncovering of the whole human genetic sequence opened up infinite possibilities for associating genetic variation with trait variation, and thus for understanding and potentially correcting genetic diseases. At last, such dreams might become reality!

Researchers have soon applied such long-awaited methods. All of these methods involve the use of sequences to search for variants, either in whole genomes, sub-genomes or in millions of fragments scattered throughout the genome, which can then be correlated with variation in phenotypic traits. For example, by comparing sequences across individuals, it is possible to characterise variants at the nucleotide level and identify SNPs (pronounced Snip) for 'Single Nucleotide Polymorphism', i.e. specific positions in the DNA sequence where variation is detected in the population. These SNPs are a formidable tool because thousands or even millions of them can be detected, as is the case, for example, in a study of embryonic stem cells and human foetal fibroblasts involving more than 12 million SNPs spread throughout the genome[70]. This type of very promising approach has been called 'Genome Wide Association Studies' or GWAS (pronounced gewaz).

However, as early as 2008, a review article by *Brendan Maher* in the journal *Nature* addressed an enigma that had emerged from the application of these methods[71]. This puzzle concerned heritability, that important quantity that is so central in evolution because it quantifies parent-offspring resemblance without which no evolution can occur. The enigma lied in a result that was surprising, to say the least. The GWAS analyses recurrently led to heritability estimates that were much lower than those obtained by the classical method measuring parent-offspring resemblance[72]. In some cases, such as the inheritance of height in humans, the heritability estimate from GWAS was 20 times lower than that obtained from the classical statistical methods. In other words, genetic variation explained only 5% of the heritability of this trait estimated on the basis of the statistical resemblance between parents and offspring!

This opened up a major question that was the focus of *Brendan Maher*'s paper, "What happened to the missing heritability?[73] This marked the beginning of a piece of literature seeking to explain that enigma. If, for example, one does a bibliographic search, one finds that the expression "missing heritability" was one of the themes of more than 1,990 articles according to the Web of Knowledge, and more than 21,400 entries according to Google Scholar[74]. Obviously, this has become a hot topic.

How to solve this enigma?

Brendan Maher's article explored several avenues to explain this enigma. You can imagine that when I read that article, I was eagerly awaiting the author's discussion of the fact that perhaps parent-offspring resemblance is based on information other than that conveyed by the DNA sequence. After all, if this were the case, higher heritabilities should be expected with the classical approaches described in **Heredity concerns** patterns of parent-offspring resemblance. It is central to biology because natural selection and evolution cannot occur without heredity. It is thus vital to study the mechanisms that produce this resemblance that involves the transmission of many kinds of information from parents to offspring. Living organisms can therefore be defined as a 'memory machine' able to collect, store, use and then transmit a wide variety of environmental information. The study of heredity is therefore the study of the different forms of information that can be transmitted across generations and affect parent-offspring resemblance. However, during the 20th century, due to the fantastic discovery of the DNA molecule and its incredible sequencic memory properties, we became blind to the existence of other types of transmission mechanisms. As a result, we have increasingly reduced heredity to its sequencic component, i.e. the sole transfer of the information encoded into the nucleotidic sequence of DNA, an attitude that I call sequencic. It is now time to re-open our views of inheritance to approach it in all its complexity. A first step to achieve this goal is to reflect about the gene concept.

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Chapter 2, compared to the estimates by GWAS, as the former methods would incorporate the effect all mechanisms responsible for parent offspring resemblance, while GWAS approaches only account for DNA sequence variation.

The first four avenues proposed to solve the problem of missing heritability[75] focused on sequencic and suggested doing even more sequencing on even more individuals, despite the fact that some studies already involved more than 30,000 individuals. One of the proposed solutions was even to redo the same kind of analysis by replacing the use of SNPs with full sequencing of thousands of individuals, which at the time would have been very expensive. However, some of the interviewed scientists disagreed with such bully approaches and suggested that serious thought should be given to the issue before embarking on such costly analyses.

The fifth explanation proposed that classical estimates of heritability might be overestimated because of the possible inheritance of epigenetic states (the subject of the next chapter), which at that time were only beginning to be seriously discussed. Parent-offspring resemblance could result from genetics, of course, but also from epigenetic inheritance. This was the explanation I was waiting for. Well, only partially, because if this explanation briefly included the potential effect of one non-genetic form of heredity, it nonetheless ignored many other forms that were well documented at the time. But this article had the merit of being not limited to a single view of inheritance.

Conventional estimates of heritability are probably overestimated

Heritability estimates by conventional methods are likely to be overestimated for several reasons[76], all of which more or less involve confusing the two meanings of the gene.

Heritability quantifies parent-offspring resemblance

First of all, heritability is only a statistical measure of parent-offspring resemblance. Heritability is therefore only a statistical term and nothing more. The value of this term quantifies the level of parent-offspring resemblance and varies from 0 to 1. When there is no transmission of the trait under study, which means that the offspring do not resemble their parents, heritability tends to 0, and the trait of the parents does not predict the trait of the offspring. In this case, the solid line in **Figure 1: The classical method to quantify parent-offspring resemblance: regressing offspring trait on parent trait**. In the theoretical case where children have exactly the same trait as their parents, the points would lie on the grey dotted line and the heritability would be 1. In real data, it can be seen that although there is some variation on the trait under study, there is a real tendency for offspring of parents with big traits to have big traits, and at least bigger than offspring of smaller parents. However, the slope of this relationship is significantly less than 1, indicating that offspring of parents with big traits tend to be smaller than their parents, while offspring of small trait parents tend to have bigger traits than their parents. This is a well-known phenomenon since *Darwin*'s cousin, *Francis Galton*, highlighted it in 1886 by establishing what is still known today as a regression. It is the slope of the regression, here the slope of the solid black curve, which quantifies the degree of parent-offspring resemblance.

is close to the horizontal. In contrast, a heritability of 1 would mean that the offspring have on average exactly the same trait value as their parents. In this case, in **Figure 1: The classical method to quantify parent-offspring resemblance: regressing offspring trait on parent trait**. In the theoretical case where children have exactly the same trait as their parents, the points would lie on the grey dotted line and the heritability would be 1. In real data, it can be seen that although there is some variation on the trait under study, there is a real tendency for offspring of parents with big traits to have big traits, and at least bigger than offspring of smaller parents. However, the slope of this relationship is significantly less than 1, indicating that offspring of parents with big traits tend to be smaller than their parents, while offspring of small trait parents tend to have bigger traits than their parents. This is a well-known phenomenon since *Darwin*'s cousin, *Francis Galton*, highlighted it in 1886 by establishing what is still known today as a regression. It is the slope of the regression, here the slope of the solid black curve, which quantifies the degree of parent-offspring resemblance.

the solid line overlaps the grey dashed line. The latter case hardly exists, except, ironically, perhaps for traits that we know are not genetically transmitted, such as the language we speak.

The animal model

The method described in **Figure 1: The classical method to quantify parent-offspring resemblance: regressing offspring trait on parent trait**. In the theoretical case where children have exactly the same trait as their parents, the points would lie on the grey dotted line and the heritability would be 1. In real data, it can be seen that although there is some variation on the trait under study, there is a real tendency for offspring of parents with big traits to have big traits, and at least bigger than offspring of smaller parents. However, the slope of this relationship is significantly less than 1, indicating that offspring of parents with big traits tend to be smaller than their parents, while offspring of small trait parents tend to have bigger traits than their parents. This is a well-known phenomenon since *Darwin*'s cousin, *Francis Galton*, highlighted it in 1886 by establishing what is still known today as a regression. It is the slope of the regression, here the slope of the solid black curve, which quantifies the degree of parent-offspring resemblance.

is partial because it exploits only part of the resemblance among relatives, that between parents and offspring. By extension this resemblance implies that a pair of relatives must be more similar than a pair of randomly chosen individuals. Another more complete method has thus been devised using the pedigrees. This method, called "animal model", exploits the resemblance between all individuals with a known relationship. If offspring

resemble their parents, it is expected that brothers will resemble each other, as well as cousins or between grandparents and grandchildren... Of course, the resemblance should decrease with the relatedness. So, offspring should look more like their parents than their grandparents or cousins. The very idea of the animal model is to weight the resemblance between two individuals by their kinship.

Information paths across generations[77]

The animal model has undoubtedly improved the estimation of heritability. But it has one important feature. The logic of introducing kinship in heritability estimation is that kinship describes the path of genetic information from the common ancestor of each pair of individuals. In the case of genetic information, the information path is well known, and this is why the relatedness between two individuals can be calculated from the family tree. A gene taken from an offspring has exactly one chance in two of being a direct copy of one of the genes of its biological father. The same applies to its genetic mother. The coefficient of relatedness between parents and offspring is therefore 0.5. Similarly, the coefficient of relatedness between siblings is 0.5. This coefficient with a cousin is 0.125... These are the values that are introduced in the animal model to estimate the heritability of a trait along lineages. And this is the only time when a little knowledge of how genetics works is accounted for when estimating heritability.

However, the family tree also corresponds to the exact path followed by any non-sequencically inherited information. For example, epigenetic information is essentially encoded in the configuration of the DNA molecule which is strongly transmitted along cell lineages and often across generations of multi-cellular organisms. This is also the case for all forms of non-genetic inheritance that we will see in this book, except for part of cultural inheritance which may follow different paths. Thus, the various systems of heredity produce pedigrees that are largely overlapping.

The coefficient of relatedness captures everything

Therefore, all type of inherited information, whether genetic or non-genetic, essentially follows the same path across the generations. Consequently, when we introduce the coefficient of relatedness into the animal model to capture the effects of sequencic transmission, this term actually captures the effects of all the information that follows the pedigree path, which includes genetic, but also most non-genetically transmitted information.

Thus, the genetic (sequencic) interpretation of the measured heritability is only one of the possible interpretations of this statistical term, and it is abusive to interpret it only in terms of sequence, which is nonetheless what we do whenever we produce heritability estimates. Of course, some authors claim that in this case they are using the pre-DNA sense of the concept of genetics, but this claim is contradicted by the fact that very often these studies include in the statistical model other variables whose role is precisely to prevent the heritability estimate from capturing non-sequencic effects. This shows that these studies are designed to extract the part of the phenotypic variation that is sequentially transmitted, and nothing else, and in effect, the next step is invariably DNA sequencing. This illustrates the extent to which we researchers are constantly playing with the two understandings of the concept of a gene. It is impossible to get out of this without clarifying the use of the term, as proposed in **Heredity concerns** patterns of parent-offspring resemblance. It is central to biology because natural selection and evolution cannot occur without heredity. It is thus vital to study the mechanisms that produce this resemblance that involves the transmission of many kinds of information from parents to offspring. Living organisms can therefore be defined as a 'memory machine' able to collect, store, use and then transmit a wide variety of environmental information. The study of heredity is therefore the study of the different forms of information that can be transmitted across generations and affect parent-offspring resemblance. However, during the 20th century, due to the fantastic discovery of the DNA molecule and its incredible sequencic memory properties, we became blind to the existence of other types of transmission mechanisms. As a result, we have increasingly reduced heredity to its sequencic component, i.e. the sole transfer of the information encoded into the nucleotidic sequence of DNA, an attitude that I call sequencic. It is now time to re-open our views of inheritance to approach it in all its complexity. A first step to achieve this goal is to reflect about the gene concept.

Chapter 2

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The heritability of language in humans

Let us take an example. Imagine a Europe-wide study of the heritability of language with many families taken from each European country. We would use an animal model because we know the pedigrees of all these people, which should lead to a good estimate of heritability. Such a study would inevitably lead to a heritability estimate of the order of 1 as everyone speaks at least the language of their parents. Estimates as high as this are almost never obtained. Yet we all know that this resemblance is produced by *social learning* (see Glossary) not by sequencic information. We all learned the language we speak from our parents in our early childhood. Indeed, in this case we know the mechanism of resemblance. This is social learning. But in the vast majority

of cases, we know nothing about the mechanisms that produce resemblance. And yet we systematically interpret it in sequencic terms.

It can therefore be concluded that non-genetic inheritance produces patterns of variation that bear a striking resemblance to those produced by genetic inheritance. This is a trap that must be avoided.

What can we conclude from the twin studies?

In the same vein, an argument often put forward to promote the importance of sequencic transmission is the study of identical twins who sometimes show surprising morphological, physiological and behavioural similarities even if they were separated at birth. Indeed, although identical twins inherit the same DNA sequence, they should diverge under the effects of the environment if separated, which often does not seem to be the case. This point is then used to argue that sequencic inheritance is very important. However, although identical twins do inherit the same DNA sequence, they also inherit a suite of non-sequencic heritable information, such as the epigenetic marks that are transmitted from parents to offspring, as we will see many examples in the following chapters. Thus, again, the strange similarities of identical twins cannot be used as compelling evidence for the importance of genetic transmission. They could even be used to argue the opposite and claim that non-sequencic information is important too.

A typical reaction

Very often, some colleagues tell me that they agree that there may be cases of non-genetic transmission, but this is anecdotal and represents at worst only a few percent of parent-offspring resemblance and therefore can be overlooked[78]. I see two major errors in this statement.

A few percent can make all the difference

First of all, to say that a few percent has no effect seems risky to me. For example, the genetic difference between chimpanzees and us is of the order of one percent. I don't think this is negligible, especially when it comes to finding a mate to reproduce.

Furthermore, it has been shown that the introduction of only a few percent variation in cooperative behaviour can change the whole evolutionary dynamic and lead to the emergence of new cooperative strategies[79]. It is therefore incorrect to say that a few percent have no *a priori* significant effect.

Missing heritability suggests a much greater weight of non-genetic inheritance

Provocatively, one could say that the amount of missing heritability sets an upper limit on the weight of non-genetic inheritance in parent-offspring resemblance. It is unlikely, to say the least, that the effect of non-genetic inheritance is just marginal, and we will see in the following chapters many examples that show that parent-offspring resemblance can also results from a wide variety of non-sequencic phenomena whose effect on heritability estimates is much larger than the few percent that is always talked about.

Conclusion

The case of the missing heritability shows to what extent our purely sequencic view of heredity is not sufficient to explain the complexity of life. Moreover, the dominant, purely sequencic vision of life is also based on the idea that the tools of modern genetics are flawless, which is obviously not the case, as no methodology in biology can claim to be flawless. To progress forward, it is now necessary to describe the ever longer and better documented list of non-genetic inheritance processes. I will present those examples, roughly following their order of emergence since the 1960s, which show the reality of inheritance processes that are not based on sequencic variation.

However, before going into the description of these many striking examples, it is necessary to take the time to introduce a fascinating and rapidly growing field of organismal biology, that of epigenetics.

Chapter 5
Epigenetics

Imagine a chef recording all his recipes in a book as he develops them, or as he comes up with new ideas to improve them. Imagine also that he passes on copies of this book to his offspring, who themselves continue adding to and refining the book as their customers' tastes change over time, for example as new ingredients are brought back from distant lands. Imagine also that they sometimes make mistakes either when applying or copying the recipes, but when they turn out to be positive, as in the case of the "*tarte Tatin*", they record them in their book. Little by little, this book would grow in size and, above all, would become more and more adapted to satisfy the tastes of the customers, bringing more and more prosperity to this family.

However, it is clear that for every meal, these chefs will never use all the recipes. Using all recipes simultaneously would only lead to chaos beyond belief, with the likely consequence that no recipe would be applied properly in the end, leading to wasted food. For each meal, therefore, they will have to choose only three or four recipes depending on the type of customer, the season and a whole host of subtle parameters. For instance, some highly active customers will need nutritious dishes, while others older or more delicate customers will need recipes that suit their delicate palate. In order for these cooks to be successful, they will need to imagine and write down a methodology somewhere in the book that will enable them to make wise choices according to the circumstances. This may take the form of a sophisticated table of contents or indexes, or other more complex procedures acting upstream of the recipes, as a sort of gateway to the wise use of this vast amount of culinary information. This methodology constitutes another form of information that is just as important as the recipes themselves, and the success of this lineage of cooks in effect depends as much on the quality of the recipes as on the effectiveness of their choice methodology according to circumstances. A prominent point is that this methodology will need to be passed on to the descendants in parallel with the recipes and may thus similarly evolve over time.

You have understood that in this metaphor of biology, the recipes represent the genes, and that the question of how to use the recipes so that each satisfies customers, obviously arises in every cell of the organisms throughout their lives. In other words, it is all very well to have a huge amount of genetic information, but they have to be used in the right way, at the right time and in the right place. This is a major issue in biology, which is the subject of what we now call epigenetics and which we will discuss in this chapter.

The origins of epigenetics

Historically, the study of epigenetics emerged from the study of development. The human organism is made up of about 200 cell types[80], such as muscle cells, liver cells, neurons, bone cells, skin cells, etc. (each of these categories containing various cell types). What is immediately striking is the extent to which these cells have contrasting phenotypes and functions, yet, as they are all formed from the division of the initial egg cell, they all have the same genetic information. The emergence during development of all these cell types from the same genetic sequence is called cell differentiation. This variation in cell types was a major puzzle that needed to be solved. How can cells with the same genetic information differ so much from each other?

The answer to this question is fairly simple. Different cell types use different parts of the genetic information to differentiate and function, much like the chefs use different recipes (genes) from their collection of recipes (genome) to make different types of meals (cells/functions). This simple idea opens up a huge and very complex field of study on the mechanisms that allow, in a given cell type, the expression to varying degrees of one set of genes and the non-expression of all other genes (which are therefore repressed or 'silenced'). For example, in rodents, genes involved in the olfactory detection of the molecules making up a bouquet of odours are expressed in only a small number of cells in the brain and are silenced everywhere else[81].

Initially, epigenetics was the science that studies the mechanisms that allow such variation in gene expression among cells of the same organism. The word epigenetics has had several meanings over its long history. Today, this science includes *the study of all changes in gene expression that do not result from variation in the nucleotide sequence of DNA and that are either transmitted during mitosis or inclusively inheritable across generations of organisms*[82].

Historically, long before the discovery of the properties of DNA, it was known that this molecule located in the cell nucleus was part of the chromatin, so called because it is easily stained for cytological studies. It was also known that it could take on two different aspects depending on the region of the nucleus, euchromatin

and heterochromatin. In fact, these two chromatin states correspond to two contrasting epigenetic states, euchromatin corresponding to expressed regions and heterochromatin to silenced regions of the DNA. These different states correspond to very different configurations of the DNA molecule within the nucleus.

Three main epigenetic domains

We saw in Erreur ! Source du renvoi introuvable, that the properties of proteins cannot be understood by studying their amino acid sequence alone, and that it is necessary to study their 3D structure. I then insisted that the same is true, on a much larger scale, for the DNA molecule. In order to understand all the memory properties of DNA, we need to understand how DNA is packaged in chromatin. In short, we need to understand its 3D structure and how it varies across regions of this immense nucleotidic chain. In fact, this could lead to defining epigenetics as the science of the heritable part of the 3D structure of DNA[83]. For this, it is necessary to integrate three major groups of molecular phenomena, (*i*) chemical modifications such as the addition of methyl or ethyl radicals at specific locations in the sequence, (ii) chemical modifications of a class of proteins called histones that play a central role in the local configuration of DNA, and (*iii*) the major role of micro RNAs[84]. We will briefly outline here some of the properties and consequences of these different processes[85].

Chemical modifications of DNA

The best documented epigenetic mechanism involves the addition of various chemical modifications to nucleotides. In many organisms, including mammals, the main such modification involves the addition of a methyl radical to some cytosine, one of the four types of nucleotides in the DNA sequence. A methyl radical is a derivative of methane, which is the simplest of the hydrocarbons because it has only one carbon atom. Its formula is CH_3.

How can the mere addition of a tiny methyl radical significantly change the configuration and therefore the use of the information encoded in the DNA sequence? To answer this question, we need to know some very general aspects of gene expression. For a gene to be expressed it must first be transcribed into messenger RNA (mRNA), which is another macromolecule in which thymines are replaced by uracils. For this transcription to take place, a molecular transcription machinery must attach to a region upstream of a gene, called the promoter. In mammals, the more methylated a promoter, the more difficult it is for this machinery to attach to the promoter. When the promoter is too methylated (i.e. when a significant proportion of the promoter's cytosines have a methyl radical), this changes the affinities between this machinery and the DNA to such an extent that transcription no longer occurs and the gene in question is therefore completely silenced. Cytosine methylation-demethylation is therefore a powerful mechanism for differential gene expression. But it is far from being the only mechanism involved.

Histone modifications

In the nucleus, the DNA is at the heart of a complex molecular structure called chromatin. First the DNA double helix is wound around nucleosomes, each consisting of eight proteins called histones, forming a kind of ball. Around each nucleosome the DNA is wound for two turns involving 146 nucleotides. The second major epigenetic mechanism involves modifications of the histones. Each of the eight histones in a nucleosome has a compact shape that leaves a chain of amino acids called a tail sprouting out of the nucleosome. At least a hundred chemical modifications of these histone tails are known. These modifications will also promote or prevent the transcription of DNA into mRNA, thus enhancing or silencing the expression of the concerned genes. It is immediately clear that these numerous histone modifications can be combined into an almost infinite number of different states for each nucleosome, thus helping to generate as many different epigenetic states for the concerned portion of the DNA sequence. One can therefore imagine the infinite diversity of possible epigenetic states.

The role of microRNAs

Until relatively recently, one of the major known roles of RNA was that of messenger, enabling it to copy the DNA sequence and transport it outside the nucleus to the cytoplasm where it is read by a complex molecular structure called the ribosome to translate it into a sequence of amino acids, i.e. a protein. RNA is most often in the form of a single nucleotidic chain, which makes it much less stable (DNA is a double nucleotide chain). However, with the fantastic development of macromolecule sequencing capabilities in the 1990s, several surprising things became apparent.

It was first discovered that a significant proportion of the DNA sequence does not code for proteins. Initially called 'junk DNA'[86], this term was quickly abandoned as it was soon realised that although these sequences are non-coding, they do play important roles for instance in epigenetics[87]. In any case, it would have been surprising if these sequences had served no purpose. Natural selection should indeed rapidly favour the loss of any sequence that is useless and yet costly to copy at each cell division. Just to get an idea of the cost

of maintaining useless sequences, consider that if you lined up all the DNA in all your cells, you would obtain a chain of some 80 billion kilometres, which would represent more than two million times the circumference of the earth at the equator. It is hard to believe that a large proportion of this chain is just useless. Today, this DNA is called non-coding DNA.

The second important discovery following high-throughput sequencing is that there is a very large variety of RNAs in cells and certain living fluids that differ greatly in length and sequence. In particular, it has been noted that many do not carry a coding sequence. As these RNAs are relatively small in size, they have been called "small non-coding RNAs (sncRNAs)". It was then discovered that some of these sncRNAs were less than 200 nucleotides long. They were therefore called micro RNAs.

The third major discovery is that at least some of these various sncRNAs play very important roles. They can, for example, affect the survival of messenger RNAs and thus their translation into proteins or not. More generally, they can affect the life span of pieces of macromolecules (DNA and RNA). They can also, on the basis of their sequencic complementarity with DNA, interact with chromatin and modify the epigenetic state and thus the accessibility of portions of DNA and thus the expression of genes present at their interaction site.

Finally, and most surprisingly, sncRNAs can be made in some cells, released into the circulatory systems, and go on to alter gene expression in all parts of the body, including the germline, which is supposed to be totally protected from outside effects (see Erreur ! Source du renvoi introuvable.)[88]. This last function highlights a major characteristic of small RNAs, allowing them to modify the information carried by gametes and thus to affect parent-offspring resemblance and thus heredity. We will see various examples in **Erreur ! Source du renvoi introuvable.** to **Chapter 10**

Randomness and mutation

After discovering all these fascinating pathways of intergenerational information transfer, it is now necessary to develop an overlooked but basic property of epigenetic marks that is linked to a recurring issue in evolutionary biology, namely that of the randomness of mutations of all types. We have seen that one of the basic principles of the Modern Synthesis is that mutations are in no way directed by the environment towards improving the adaptation of organisms. Unfortunately, this principle is often simplified into saying that mutations occur at random, which does not mean the same thing. But what exactly is the case? This is what we will look at in this chapter.

Epigenetic marks are mutagenic…

The starting point that led me to think about the issue of mutation randomness was the fact that epigenetic marks, such as the presence of methyl radicals on cytosines, destabilises DNA and greatly increases the mutation rate of methyl-cytosines into thymine, another base of the DNA sequence. This, therefore, has the potential to generate point mutations whereby a cytosine is replaced by a thymine. Some articles have, for example, subheadings entitled "Methylation is mutagenic". For example, studies in humans suggest that cytosine methylation is responsible for 30-40% of point mutations in the human germline. Combining the results of several authors, cytosine methylation would increase the probability of cytosine mutating to thymine by a factor of about 20,000. This is such a considerable factor that it seems very unlikely that it is a negative collateral effect of a process selected in another context (in this case DNA methylation, which is involved in the regulation of gene expression). What then could be the function of a process that destabilises the fidelity of sequencic transmission to such an extent?

This is what we addressed in a 2019 paper. We proposed a mechanism by which such mutagenic power of DNA methylation, and more generally of epigenetic marks, might have provided a real evolutionary advantage by accelerating the sequencic engraving of the initially plastic responses to environmental conditions that prove to be very persistent. We have given this mechanism the explicit but unmemorable name of *epigenetically-facilitated mutational assimilation*.

Genetic assimilation

The idea of *genetic assimilation* (see Glossary) was proposed by *Conrad Waddington* following a series of experiments in *Drosophila* showing that following an environmental stress triggering an initially plastic response, this response tends to become heritable (and therefore non-plastic) after a certain number of generations under the effect of this stress. It was therefore as if, after a few dozen generations, characters initially developed in a plastic manner in response to a given environment became ‘genetically’ engraved, hence the expression 'genetic assimilation'.

Genetic or epigenetic assimilation?

However, it should be noted that in this expression the term genetic was understood in its pre-DNA sense, as 'that which is transmitted', without prejudging the mechanism responsible for this transmission. In particular, while Waddington's experiments undoubtedly demonstrated that the initially plastic trait became inclusively

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heritable, they did not at all show that this necessarily implied a sequencic change. In effect, there was nothing in these experiments to suggest that what he observed at the phenotypic level resulted from a change in the DNA sequence. Given that Waddington had only worked over a few dozen generations —which was already a real challenge —he in fact most likely documented an "epigenetic assimilation" because the only thing his experiments really showed was that an initially plastic trait became inclusively inheritable within a few generations. This is equivalent to what *Mary Jane West-Eberhard* called "genetic accommodation" whereby a trait can be made heritable without necessarily involving encoding in the DNA sequence. Our paper proposed that, under certain conditions to which we will return later in this chapter, this process could go as far as sequencic engraving, *if the environmental stress persists over many, many generations*.

And the Modern Synthesis assimilated genetic assimilation

It has always puzzled me that the idea of genetic assimilation has finally been 'assimilated' by the Modern Synthesis, as this mechanism is strongly reminiscent of the much-rejected idea of inheritance of acquired traits. If you think about it, Waddington's mechanism proposes that within a few dozen generations under a given constant environmental stress the initially plastic response to stress can become heritable. In fact, what has allowed the idea of genetic assimilation to be assimilated is the relative slowness of this phenomenon. Moreover, the classical interpretation of this phenomenon is that there would pre-exist some neutral and hidden sequencic variation (usually called standing genetic variation) that would be somehow revealed by the environmental stress. Natural selection would then have the time to act over the few dozen generations of Waddington's experiments to retain only those variants that happen to be, I would like to say 'miraculously', favourable. So genetic assimilation would be just a special case of natural selection. This is how the Modern Synthesis has managed to see no major contradiction in genetic assimilation. This is also how I understood it until a few years ago.

Epigenetics as a hub towards sequencic engraving

A striking result on which we have built our reasoning is that all mechanisms of non-genetic heritability seem to involve some epigenetic change. It is as if epigenetics was the backbone or hub towards which most non-genetic inheritance processes would converge. Then, as epigenetic marks destabilize the DNA, over the course of many generations, this would generate sequencic variation *in the parts of the DNA concerned by the accommodation to the environmental change*. This would lead through natural selection acting on this newly produced variation, to sequencic engraving. In a way, epigenetics would be the conductor of the orchestra made up of all the genetic information. In effect, while it is very useful to have all the sequencic information (the recipe book), it is important to use it wisely. We shall see in **Chapter 16** that this epigenetic conductor is itself under the control of the brain.

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With *Arnaud Pocheville*, then based at the University of Sydney in Australia, we modelled this idea and were able to show that such a mechanism could accelerate the transfer of epigenetic encoding to sequencic encoding by a factor of the order of magnitude of the mutagenicity of the epigenetic marks, i.e. about 20,000 times. *This is what we called the epigenetically-facilitated mutational assimilation*.

But the story does not end there, as epigenetics interacts strongly with another major source of mutation, namely transposable elements.

... and interact with transposable elements

In parallel, we have been interested in another major phenomenon that can affect both the expression of certain genes and the appearance of mutations of all types. In fact, not only can the presence of epigenetic marks affect the stability of DNA, but epigenetic marks are themselves in strong interaction with the activity of transposable elements. Transposable elements are mobile DNA sequences discovered in maize by *Barbara McClintock* at the Cold Spring Harbor Laboratory on Long Island in the USA in the 1940s. This is one of the great genetic discoveries of the second half of the 20th century. There are a variety of transposable elements that differ, among other things, in the way they duplicate. Transposable elements exist in almost all living organisms. They seem to be able to invade the genome of an entire species through a process of colonisation from a local population, and can represent a large portion of the genome (about 15 to 22% in Drosophila, 40% of the genome in humans, and up to 90% in wheat). To give an idea of the prevalence of transposable elements, in humans, more than three million human sequences are derived from transposable elements, but only a few hundred of these have retained transposition capacity. The universality and mobility of transposable elements suggest that they play an important role in genome evolution and plasticity

The activity of transposable elements is under epigenetic control

The activity of transposable elements is strongly modulated by epigenetic processes (involving methylation, histone modifications or small RNAs) which are themselves affected by environmental factors. There are

several hypotheses (not necessarily mutually exclusive) explaining the interaction between transposable elements and epigenetics. In particular, the targeting of epigenetic modifications to transposable elements could be a consequence of the *exaptation* (see Glossary) of transposable elements as platforms for chromatin modification, in which case the epigenetic regulation of transposable elements could be a consequence of genome defence and regulation. As a result, environmental stresses can trigger transposition activity, either directly or through their effects on epigenetic marks associated with transposable elements. It can be said that in most cases the mobility of transposable elements is inhibited by epigenetic marks that block their replication. However, this targeting of epigenetic marks on transposable elements also affects, as if by ricochet, the genes close to these transposable elements —with which they become partners in a kind of "transposable-element-gene duo"—, thus affecting their expression level. Beyond their important mutational effects, by duplicating themselves in the genome, transposable elements can thus affect the general functioning of the genome, among other things by regulating and controlling the activity of genes in the neighbourhood of their insertion point. Thus transposable elements affect gene activity in three different ways.

- First, by attracting strong epigenetic marking around their insertion point, they affect the epigenetic marks, and therefore the expression, of the genes with which they are in duo. It should be noted that the epigenetic marks around transposable elements can be modified by stresses bringing back their mobility, hence modifying the expression of the genes around the new insertion point.
- On the other hand, as the sequence of many transposable elements carries regulatory elements of response to the environment, their presence will directly modulate the expression of the genes with which they are in duo according to the environmental context. They therefore play a central role in the response to environmental changes.
- Finally, by their mobility within the genome, transposable elements can generate significant sequencic changes in the genome. Their mutagenic potential is thought to increase the average point mutation rate by several tens of thousands of times.

A great generator of inclusively heritable variation

Thus, the presence of transposable elements in one area of the genome can on the one hand durably modify the expression of the surrounding genes due to the strong intervention of persistent epigenetic marks inhibiting their mobility, and on the other hand generate genetic (sequencic) variation in the whole genome as a result of their mobility. Both types of variation can affect the phenotype either negatively for individuals (e.g. they are implicated in various diseases) or positively at the population level by generating variation that is inclusively heritable and therefore open to selection. In other words, while at the individual level these changes can often have negative consequences, at the population level transposable elements generate inclusively heritable variation on which natural selection can act, thus favouring the adaptation of populations to their environment.

Interactions between epigenetics and transposable elements thus constitute a real engine for the creation of phenotypic variation (targeted to specific portions of the genome) that can be inherited either sequentially or epigenetically *in response to environmental stresses*, and are thus an important factor in evolution. Such a generator of genetic and epigenetic variation can in particular explain changes in mutability within the genome following environmental stresses. Several authors have emphasised the existence and importance of such generators of inclusively heritable variation involving the joint action of genetic and non-genetic processes in the ability of natural populations to adapt to ongoing global changes under the influence of human activities.

Epigenetically-facilitated mutational assimilation

We can now synthesize this. It appears that the effects of environmental stresses can affect the expression of specific genes involved in the response to stress and affect the activity of transposable elements, two major characteristics that each have the capacity to increase the sequencic mutation rate by tens of thousands of times, which is anything but negligible.

An information transfer pathway acting over many generations

The epigenetic changes affecting the expression of genes specifically involved in the response to an environmental stress in fact have two functions taking place on two very different time scale:

- First, these epigenetic marks, which we have seen target very precise portions of the DNA, enable the individual to adapt to the current environment by finely regulating the expression of the genes involved and leading to the phenotypic response to the environmental challenge. This response is rapidly established under the effect of environmental change. This process is known as phenotypic plasticity, the ability to modify the phenotype in response to the environment.
- Second, by being inherited, those epigenetic marks lastingly affect the mutability of the concerned genes that happen to be the genes involved in the accommodation to the specific environmental change. These epigenetic marks can also affect the activity of neighbouring transposable elements, which can further increase the

mutability of the concerned regions and thus the potential generation of sequencic variation. In other words, epigenetic marking would differentially mark portions of the genome for mutation, i.e. for the generation of sequencic variation and thus for the multigenerational exploration of new genetic possibilities. Far from being a cost in terms of evolution, this may on the contrary constitute a major evolutionary benefit because the sequencic variation thus generated concerns the genes actually involved in the accommodation to the specific environmental stress, a variation then open to natural selection.

This is *epigenetically-facilitated mutational assimilation* that is more than just a special case of natural selection on initially neutral and hidden genetic variation suddenly revealed by environmental change. According to our view, genetic assimilation appears as *a genuine mechanism for manufacturing sequencic variation in the parts of the genome concerned by the accommodation to the specific environment*, variation which is then open to natural selection. This mechanism calls for several important comments.

Random mutations in environmentally targeted areas of the genome

First, with epigenetically-facilitated mutational assimilation, the fundamental axiom of the Modern Synthesis that *mutations are not influenced by the environment in an adaptive direction* remains 100% valid. However, it is the simplified phrase traditionally used to simplify this axiom "mutations are random" that appears incorrect. With epigenetically-facilitated mutational assimilation the mutations generated following a lasting environmental change are indeed not influenced in an adaptive direction by the environment (the axiom of the Modern Synthesis therefore remains valid), but the parts of the genome where the mutation rate increases are actually targeted by the environment. *This is because epigenetic changes and the activity of transposable elements are themselves targeted by the environment*. There are therefore two independent scales where randomness can be expressed, that of regional portions of the DNA, and that of the local change of sequence itself. Only the second scale is unaffected by the environment, whereas the regional scale is clearly targeted by the effects of the environment in the sense that it is precisely in the portions of the DNA concerned by the accommodation to the environmental challenge that the mutation rate changes.

A necessarily slow process…

Second, even if the magnitude of several tens of thousands of increase in mutation rate seems enormous, it does not mean that epigenetically-facilitated mutational assimilation (i.e. the sequencic engraving of the adaptation) takes place in a few generations. A rough calculation predicts that such a process must take hundreds, if not thousands, of generations to become effective. Although the calculation proposed in the last note is very crude, the important point is that we should not expect epigenetically-facilitated mutational assimilation to take place very quickly, and certainly not in only a few tens of generations. And in fact, evolutionary logic even leads us to believe that this slowness is integral to the process (see below).

… which could be involved in domestication

We were certainly not the first to think about this type of genetic assimilation where the environment can be involved in generating genetic variation in the sections of the genome involved in the response to the environment. For example, one of the earliest papers on the subject dates back to 1983 in which *Hugh Iltis*, then Professor of Botany at the University of Wisconsin, formalised a scenario for the domestication of maize from teosinte, an annual plant from Central America. This remarkable scenario integrated several previous hypotheses and involved the major and massive effect of what he called a catastrophic epigenetic sexual transmutation that occurred some seven millennia ago.

Similarly, the whole literature on transposable elements claims that the environment can generate inclusively heritable variation. Regarding the idea that the environment can generate variation in certain regions of the genome, *Eva Jablonka* and her collaborators had modelled this idea without proposing a molecular mechanism. Similarly, *Michael Skinner* also foresaw and proposed the existence of such phenomena. Furthermore, researchers working on the domestication syndrome of vertebrates proposed that the stress induced at the beginning of domestication must have caused alterations in the methylation patterns of developmental genes expressed in the neural crest (the part of the embryo that will become the central nervous system), epigenetic changes that could have been fixed in the form of genetic variants to explain recurrent behavioural resemblances in the many domesticated fish, mammals and birds.

The different systems of inheritance interact with each other

This chapter thus introduced a particularly important point, namely that the different systems of inheritance (which we will summarise in **Chapter 15**) do not operate independently of each other. On the contrary, they interact and influence each other. For example, the central idea of epigenetically-facilitated mutational assimilation is that the molecular memory represented by epigenetics states interacts over the long term with sequencic memory, in a way that can potentially considerably accelerate the genetic encoding of initially plastic responses to environmental characteristics that persisted for hundreds or thousands of generations.

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Three interacting mechanisms

Together, these three processes participate in establishing epigenetic marks that generate variable epigenetic states that affect the accessibility of the relevant portion of DNA to the cellular machinery that enables gene expression. Whereas chemical modifications of DNA and histones concern processes taking place close to the DNA molecule in the nucleus itself, small RNAs can affect the state of DNA in the nucleus but also affects post-transcriptional processes in the *cytoplasm* (see Glossary) of the cell, but also in other cells of the body. Epigenetic processes can occur in all cellular compartments and have the potential to affect all facets of cellular metabolism, and thus of organisms. On the other hand, although for purely technological reasons these three major types of molecular processes are most often studied separately, the epigenetic state of a cell cannot be fully understood without an approach that integrates all three components simultaneously. It is known, for example, that there are correlations between histone marks and the methylation of the relevant sequences. There is probably a certain amount of redundancy between these different types of marks, which also allows these epigenetic states to be engraved in a more or less durable way throughout the life of an individual, but also across generations.

Thus, the rise of high-throughput sequencing at the end of the 1990s led to a series of major discoveries that showed the extent to which the study of the DNA sequence alone was not sufficient to explain the complexity of life.

Epigenetics, the science of the heritability of the 4D structure of DNA

At the very beginning of this section I suggested that, in its current acceptation, epigenetics could be redefined as the science of the 3D structure of DNA. In fact, this definition would be incomplete because it neglects the important fourth dimension of the temporal dynamics of gene expression. The translation of mRNAs into protein is strongly regulated by a range of factors, some of which are under environmental control (for more details on the different types of RNA currently identified, see **Box 2**).

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Box 2: Some of the various types of ARN.

Unlike DNA, there is a wide variety of RNAs, each with very specific functions in the functioning of somatic and germline cells. Here is a non-exhaustive list of the various identified types of ANRs (in alphabetic order).

- **22G-RNA**: A form of siRNA. RNAs that have a length of less than 22 nucleotides (nt) and possess a triphosphorylated 5 guanosine (G), comprise the most abundant type of endo-siRNA in *C. elegans*[89].

- **lncRNA**: Long non-coding RNA: that bind to the chromosomes and alter their three-dimensional structure [90].

- **miRNA**: microRNA. A generic term regrouping any kind of RNA involving a few tens of nucleotides.

- **mRNA**: messenger ARN. The category of RNAs that are made in the nucleus to carry the DNA sequence to the cytoplasm at the level of the ribosomes where it is translated into a sequence of amino acids, i.e. in a protein.

- **piRNA** (or 21U-RNAs): Piwi-interacting RNA. Alternatively termed 21U-RNAs. A class of sncRNAs characterized as being 21 nucleotides in length with a 5 uracil, therefore alternatively termed 21U-RNAs [91]. To date, over 15,000 unique 21U-RNAs have been identified, most of which are expressed in the germline to regulate endogenous targets[92].

- **RNAi**: RNA interference. A biological process by which specific micro (miRNA) or small interfering RNAs bind to messenger RNAs (with a complementary sequence). The resulting double-stranded RNA is then targeted for degradation, resulting in the destruction of the messenger RNA that has a matching sequence. The small RNA intermediates of this process can also modify gene expression in the nucleus: Some sncRNAs appear to act mainly by biding to mRNA and thereby disrupting their translation into peptides.

- **rRNA**: Ribosomal RNA. A type of non-coding RNA that is the primary component of ribosomes, essential to all cells.

- **siRNA**: small interfering RNA, can be of exogenous or endogenous origin. These RNAs bind to various forms of RNAs (particularly mRNAs), which leads to the degradation of the corresponding ANR. It is involved in the regulation of gene expression unfolding at the level of the cytoplasm. (See RNAi above).

- **Small RNAs**: A generic term to qualify all small RNAs, usually less than 200 nt) and non-coding.

- **sncRNA**: Small non-coding ARN (usually less than 100 nt).

- **tRF: tRNA Fragments**. Portions of tRNAs that play various functions in the cell.

- **tRNA**: transfer RNA: the RNAs that brings the amino acids at the ribosome in front of the right codon during protein synthesis. It has an anti-codon that pairs with the codon of the mRNA. About 76 to 90 nucleotides in length.

- **tRNA fragments** (approximately 28-34 nt): predominantly derived from the 5' ends of tRNAs[93]

- **tsRNA**: a modification of tRNA fragments[94].

Synonymous codons affect the dynamics of protein synthesis...

A first factor concerns synonymous codons. The genetic code is degenerated in that many amino acids can be coded by several codons that are called synonymous because they lead to the same protein in terms of its amino acid sequence. However, for a given organism, one of these codons is predominantly used to encode the specific amino acid, the other codons being much rarer. Rare synonymous codons have been found to significantly affect the function performed by the protein in question[95]. Although synonymous, these rare codons slow down, or even stop prematurely, the translation of messenger RNAs (Box 2) into protein in the ribosomes[96]. In addition, it can influence the folding of the concerned protein, which then affects its biological function. This slowing down can result in the protein indeed being produced, but not with the right shape or at the right time to fulfil its function. For example, in *Escherichia coli*, the disruption of the kinetics of synthesis of a highly expressed protein induced by a rare synonymous codon can decrease the efficiency of translation and reduce the fitness of the bacterium[97]. In other words, although synonymous codons code for the same protein sequence, the temporal dimension of protein synthesis may itself change the biological function of proteins, introducing another source of phenotypic variation.

Apart from suggesting that the use of different synonymous codons is itself under selection pressure, this property highlights the importance of the kinetics of protein synthesis and the fact that different kinetics generate variation due not to the 3D shape of the DNA but to a fourth dimension, that of the dynamics of protein synthesis. Of course, codon usage biases are part of sequencic heredity, but this phenomenon has the merit of introducing an important nuance in the classical vision of genome functioning and in the equivalence between resemblance and sequence.

*.... as well as numerous mRNA and tRNA modifications (see **Box 2**)*

The regulation and efficiency of translation of mRNAs (messenger RNAs) into protein is also strongly influenced by numerous modifications of mRNAs, or tRNAs (transfer RNAs) which, by affecting the initiation of translation and the dynamics of codon-anticodon interactions, accelerate, stop or slow down protein synthesis, thus affecting the phenotype[98]. Beyond the fact that this regulation plays a major role during cell proliferation[99] and differentiation[100], the resulting variations in kinetics can affect the phenotype and in particular the health (i.e. fitness) of organisms, as for example in the proliferation of cancer cells[101].

As these modifications are often influenced by environmental stresses[102], these translation regulatory mechanisms are therefore involved in phenotypic plasticity. These mechanisms produce variation in functional gene expression that is completely independent of variation in the DNA sequence. Although there does not appear to be any paper to date reporting the transmission of mRNA translation regulatory states across generations, it is likely that this absence only stems from the fact that these processes have been described only recently and I would be very surprised if some of these processes did not turn out to be transmitted. At the time of writing there is a large international project to study the dynamics of genome conformation in space and time (3D and 4D)[103] that should bring new information soon.

Thus, anticipating such discoveries, we can propose a more complete definition centred on the functional nature of epigenetics, which would be *the science of the part of the 4D structure of DNA that is transmitted either during mitosis or across generations of multicellular organisms. This definition naturally includes all the 3D aspects linked to the configuration of the nucleic acids within the chromatin, but also all the components of the dynamics of gene expression*, which, via its effects on the efficiency of the translation of mRNAs into protein, can influence phenotypic fitness. In other words, if we focus on the functional nature of epigenetics, we can say that *it is the science of the heritability of the 4D structure of DNA*[104].

Epigenetics and heritability

The definition of epigenetics always incorporates two important elements: not only are epigenetic states reliably transmitted along cell lineages, but, in addition, they can also be transmitted through sexual reproduction either through gametes or other pathways[105]. It is this aspect of epigenetics that is of particular interest to us in this book.

A story of toadflax

Historically, one of the earliest examples of epigenetic inheritance was documented in toadflax (*Linaria vulgaris*)[106]. This roadside and wasteland plant has bilaterally symmetrical flowers due to a strong dorsoventral asymmetry that resemble the flowers of snapdragon (*Antirrhinum majus*), a plant often cultivated for its beautiful flowers. However, the father of taxonomy, *Carl von Linnaeus*, saw individuals with radially symmetrical flowers appearing in toadflax natural populations, which he termed '*peloric*' (Greek for monster or marvel). The appearance of these peloric flowers fascinated *Linnaeus*, as he believed that the world had been created in its current state, a fixist view that was the dominant conception of his time. *Linnaeus* thought that plants with peloric flowers resulted from interspecific pollination.

This floral polymorphism, which has long been used as a classic example of a how natural mutation can affect the phenotype, has however been shown to be due not to the presence of a mutation, but to the presence of an atypical methylation pattern on the *Lcyc* gene which is involved in floral symmetry[107]. In peloric forms this gene is highly methylated and therefore silenced, and the inheritance of this trait is due to the fact that this methylation state is passed on to the offspring over the generations. This example illustrates how non-genetic and genetic inheritance can produce very similar patterns of variation. This leads us to be wary of a purely sequencic interpretation of parent-offspring resemblance. It also shows that transmitted epigenetic variation can generate population patterns of variation that, like sequencic variation, are subject to natural selection.

Transmission during mitosis

Epigenetic states are transmitted during cell division (i.e. mitosis). This is known as mitotic heritability or heredity, or cell memory[108]. This characteristic is fundamental for the proper development and functioning of an organism, as it avoids having muscle or bone cells in the liver or brain for instance. With rare exceptions, once differentiated, a cell produces cells of its own type. This characteristic is widely used in medicine. For example, one can take skin cells from an individual and then cultivate them in the laboratory to make skin grafts. Conversely, the consequence of mitotic memory is that one cannot easily make tissues other than skin cells from samples of skin cells.

The epigenetic states produced by the various types of epigenetic marks are copied during cell division. DNA duplication involves several steps, the first of which is the opening of the DNA into two strands (each corresponding to one of the legs of the DNA ladder). This can be visualised as a zipper that would be closed in its normal working state but would open during duplication, releasing the two complementary strands. Once separated, each strand serves as a template for the reconstruction of a new complementary strand identical to the one from which the old strand has just been separated, and vice versa with the other strand. What is less well known is that the epigenetic marks present on the old strands are also reproduced on the newly made strand, thus transmitting the pre-existing epigenetic state on the other strand. This is why the division of a liver cell will always result in two liver cells of the same type as the initial cell.

This is true for all differentiated cells in our body. But there is a category of undifferentiated cells called stem cells that, being undifferentiated, can produce just about every cell type in the body. This is why these stem cells are of great medical interest, because they can theoretically make all types of tissue.

Heritability in sexual reproduction

A major discovery in biology over the last 20-30 years is that certain epigenetic states are often transmitted for surprisingly high numbers of generations along lineages of multi-cellular organisms. We will see the great diversity of molecular processes involved in the five chapters that follow.

Such intergenerational transmission of epigenetic states poses a major conceptual problem. In Erreur ! Source du renvoi introuvable., we introduced the Weismann barrier that the environment cannot change the information transmitted across generations. This idea, which is more than a century old, has been reinforced by the discovery of the existence of waves of demethylation-remethylation occurring at several points in sexual reproduction, in particular during meiosis and after fertilisation. This idea follows an irrefutable logic. Indeed, ageing results from the accumulation of mutations and potentially deleterious epigenetic marks. This has led to the establishment of two basic principles.

- The germline must somehow escape such negative effects, otherwise deleterious mutations during life would accumulate over the generations. This is the Weismann rule (or barrier).
- This rule requires a mechanism to erase from the germline all epigenetic marks that have managed to pass through this barrier and that have accumulated during the stresses of life. Thus, reproduction must reset all the counters to produce offspring free of all these deleterious environmental effects accumulated by the parents, and the waves of demethylation-remethylation provide the molecular mechanism for this reset.

The logic of these two rules is sound, for without them, life could not persist. It is because non-genetic heredity seems to violate these two basic principles that it took several decades for its existence and especially its omnipresence to be accepted[109]. However, these two rules need to be nuanced because it is now indisputable that the so-called Weismann barrier is not as impermeable as usually claimed, as some epigenetic marks seem to pass through and escape the mechanisms for resetting epigenetic marks during reproduction. These are probably exceptions that prove the rule[110].

Therefore, the environmental effects that would seem to pass through these many filters are engraved in a very resistant epigenetic way and would probably be the result of a history of selection that favoured their maintenance across generations. It is this type of reasoning that led me to put a question mark in Erreur ! Source du renvoi introuvable.: there must be mechanisms by which certain environmental effects —*a priori* those resulting from environmental changes that last beyond one generation— are transmitted to subsequent generations. We will address these issues in the third part of this book.

Epigenetics, a world to explore

The discussion on the respective roles of genes and environment in development has always been very active and used to be summarised in the opposition nature *versus* nurture. Since those days we have discovered the fascinating properties of epigenetics, which shows that not only are there non-genetic mechanisms of inheritance, but that these are not necessarily based on learning in the environment. In this chapter, we have only scratched the surface of this fascinating area of modern biology, which is in fact at the heart of this book. Indeed, I have limited myself here to specifying the aspects of epigenetics that are necessary to understanding the examples of non-genetic inheritance presented in the rest of this second part.

Over the past 20 years, the field of epigenetics has been one of the most dynamic and discovery-producing areas in all of biology. This field is so vast that it would take books to try to give a general picture. The discoveries made by epigenetics can sometimes lead to situations that reveal a change in mentality that does not occur at the same speed in all minds. For example, the anecdote I experienced in Toulouse during the annual colloquium of TULIP, the Laboratory of Excellence that I co-directed with *Dominique Roby* for 10 years. We had invited a specialist in tomato genetics. The first question at the end of his interesting talk on the genetics of plant yield was something like 'I was surprised that you didn't mention epigenetics'. The speaker then metamorphosed and said something like "At one time everything was genetic, now it's all about epigenetics". He almost said that before science we attributed misunderstood processes to gods, and now we say it's epigenetics. I remember the roar of the room visibly shocked by this answer. This person was producing tomato varieties adapted to different environmental conditions. Obviously, from his standpoint, an adaptation written into the DNA sequence is so much more persistent he can guarantee the outcome, whereas the same capacity carried by particular epigenetic states would be far too labile to guarantee the outcome. But we shall see that this is precisely the point of epigenetics, that it allows an adaptation that can be passed on accurately, but nonetheless in a way that remains *reversible*, which is not the case with adaptations inscribed into genes.

All the features described in this chapter make epigenetics a subtle molecular mechanism of accommodation to current environmental conditions. By finely regulating gene expression, epigenetics is involved not only in development, but also in fine-tuning the functioning of the organism to the conditions encountered throughout life. However, while most biologists will tell you that epigenetics is a developmental (i.e. intra-generational) process, we must admit that it is also a process of inheritance (i.e. intergenerational)[111].

Finally, this chapter has illustrated one of *August Weismann*'s key ideas, namely that in order to study evolution, we must also study development. This major idea is at the very heart of this book, and this chapter is an illustration of it. It is not for nothing that this idea is one of the leitmotifs of the scientific movement that has been calling for the modernisation of the Modern Synthesis for more than two decades[112].

Chapter 6
Inheritance of Parental Behaviour in Mammals

In 1980, I spent several weeks with my wife and my one-year-old son and a couple of bird-loving friends on an uninhabited islet called Burhou off the Channel Island of Alderney. This islet is surrounded by the tidal currents of the Raz Blanchard which can reach 12 knots, which I believe is the French record and which makes access to it particularly tricky. I wanted to count all breeding seabird pairs on this archipelago. The discovery of the egg containing nests was a great pleasure that was reminiscent of our childhood joy in searching for Easter eggs in our parents' garden. I took an exaggerated number of pictures (silver photos and thus expensive) of these nests, so much so that I needed to engrave these magnificent moments in my memory.

It was during that census that I was confronted with a strange phenomenon. Surprised by my discreet arrival, an incubating shag (*Phalacrocorax aristotelis*) threw itself down the cliff from its nest and really gave me the impression that it had a broken wing. I rushed over to try and catch it and see what I could do, but as I got closer it flew farther and farther away from its nest, still with its apparently broken wing and showing a great deal of feverishness, and finally flew off in the most normal way towards the open sea. I then realised that it had really fooled me. I had heard of this effective behaviour, which consists of becoming very visible while mimicking a broken wing to attract any would-be predator away from the chicks, but had never experienced it yet.

I was again a victim of the perfection of this behaviour, even though I already knew it. This time it was the Least Sandpiper (*Calidris minutilla*) a tiny shorebird nesting on Middleton Island in the Gulf of Alaska where I worked for over ten years with my students. The amazing thing is that it is so hard to resist our uncontrollable hunting instinct, and once we realise the subterfuge it is too late to hope to locate the nest, and we feel foolish every time for having been tricked again! This is one of the countless types of parental care that exist in many animal species by which parents expose themselves dangerously to protect their offspring from predators. Such parental care is costly in terms of time and energy and can involve real risk-taking. As such behaviour exist in first breeders, they are considered to be genetically transmitted.

Similarly in mammals, after giving birth, females literally fall in love with their pup(s). Throughout infancy, mothers are focused on their young. They caress them, warm them, nurse them; in short, during this relatively long period of life, all their activities are focused on their young. This profound behavioural reorganisation, which plays a central role in the persistence of lineages over generations, is often called parental instinct or love. Depending on the species, this phenomenon is stronger in mothers than in fathers, but it can be the opposite in other species, and it is often equally real in both parents. Of course, the human species is no exception. It is striking to see how young parents no longer talk about anything other than their baby's development and seem, for a time, disconnected from any other reality.

While it is relatively easy to imagine the selective advantages of such parental behaviour through their positive effects on offspring survival, the question arises as to the neurophysiological mechanisms involved in this parental behavioural syndrome. How are these changes triggered and how is such parental behaviour transmitted across generations? This is what we will discuss in this chapter.

Parental care varies within populations...

In this context, it is striking that there is intra-population variation in this behavioural reorganisation. This variation concerns both the form taken by the care and its intensity. For example, I have regularly observed this phenomenon in black-legged kittiwakes (*Rissa tridactyla*), which I have been studying for almost 40 years. Some pairs may simply forget to incubate their eggs. In this bird, these neglectful parents are often inexperienced parents, but this is not always the case. Similarly, in mammals, parental behaviour varies greatly from pair to pair in a recurrent way throughout life. Some parents are totally focused on their offspring (the so-called “mère poule” in French), while others are seemingly less so, or more rarely tend to actually neglect their young. In rodents, there is also a wide variation in the level of maternal care within a population[113]. In humans, at the extreme, some parents do not show this parental instinct, which can, fortunately rarely, lead to real tragedies resulting in abuse or even the death of the child.

This variation is all the more surprising that it can be transmitted from generation to generation, with offspring reproducing the behaviour of their parents so that the behavioural variation persists in the population. The offspring of careful parents tend to be careful with their own young, while the offspring of careless parents tend to neglect their young and so on down the generations. This is therefore a typical example of parent-offspring resemblance revealing the heredity of parental behaviour.

The classic reaction to such a situation where differences among family lines persist across generations is to assume that these lines must differ genetically. However, in rodents, which are the classical model for studying the biological basis of this inherited variation in parental behaviour[114], several biological facts rule out this purely genetic interpretation.

...that cannot be explained by genetic variation

First, if immediately after birth, pups are taken from a nurturing (or careless) mother and adopted by a careless (or nurturing) mother, when adult, the adopted daughters behave like their adoptive mother, not like their genetic mother. For example, in 1963 *Victor Denenberg* and *Arthur Whimbey* of the University of Lafayette in Indiana were the first to show that the handling of rats pups just after birth had consequences for their future adult behaviour[115]. Using cross-fostering experiments (as many other studies since[116]) they showed that only the handling status of the adoptive mother and not that of the biological mother mattered. On the other hand, other studies showed that if such cross-fostering experiments are carried out later in life, then the daughters resemble their biological mothers (who had cared for them immediately after birth, before the adoption). Thus, something happens very early in life that permanently shapes the young females to resemble, in terms of maternal behaviour, the female who cared for them immediately after birth. This feature eliminates any purely genetic explanation for such behavioural variation.

An acquired but nonetheless transmitted variation

Furthermore, a family line of careless females can easily be initiated in the laboratory by regularly separating the pups from their mothers immediately after birth. These youngsters are then raised by mothers who, although providing normal maternal care, appear careless to their babies as they are regularly frustrated from maternal care during manipulations. Then, as with natural variation, the resulting adult females raised by apparently careless mothers become themselves truly careless mothers to their daughters who in turn become careless mothers, and so on for many generations. The existence of a period during which young depend on parental care is conducive to the development of such parental effects and the transmission of environmental information.

Thus, if very young females are deprived of parental care, once they become adults, they neglect their young, which in turn will produce careless daughters, and so on. Careless mothers form the environment for their young, which leads them to reconstruct the same type of behaviour as their mother. Note that this phenomenon is initially triggered by an environmental factor (in this case, the removal of the young pups from their mother, temporarily frustrating them with maternal care) that leads females to acquire a new behaviour (carelessness towards their pups), thus starting a family line of careless mothers over many generations.

Each of the above two features rules out a purely genetic explanation for the existence and maintenance of inter-lineage variation in maternal behaviour. This then raises the question of what might trigger such behavioural variation in a way that is transmitted along a family line.

Mechanisms of maternal behaviour inheritance

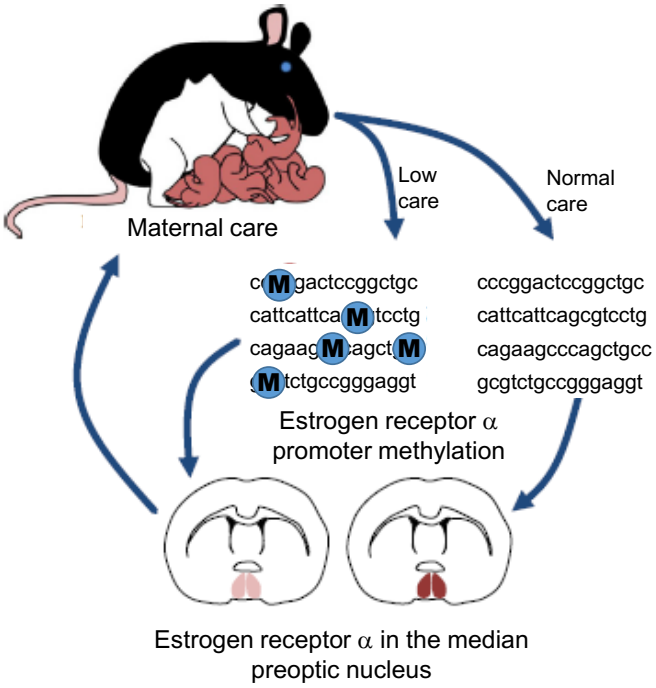
To understand this inheritance of parental behaviour, one can study the infra-individual mechanisms (Tinbergen's responses 1 and 2, see Erreur ! Source du renvoi introuvable.), or the supra-individual mechanisms (responses 3 and 4). Given the original characteristics of this form of heredity, it was first necessary to understand the infra-individual mechanisms leading to parent-offspring resemblance. And it is indeed in this area that we have learned the most since 1963.

A milestone was the paper by *Darlene Francis* and colleagues at McGill University in Montreal, Canada, whose title was particularly provocative at the time of publication in 1999, as it could be rephrased as "Non-genetic inheritance of maternal behaviour in rats"[117]. This article showed that the mode of transmission is not based on sequencic but rather epigenetic variation, and suggests avenues of research by showing the existence of important differences in the hypothalamus and hippocampus (two brain regions) of young rats raised by nurturing or neglectful mothers.

Nine years later, *Frances Champagne* of Columbia University in New York reviewed the state of the art in this transmission in a 2008 review article[118]. This mechanism, summarised in **Figure 6**, is based on the fact that the level of maternal care has a lifelong effects on the epigenetic state of genes coding for sexual hormone receptors in specific brain regions of young female rats.

For instance, in females reared by nurturing mothers the promoter of the gene encoding the α oestrogen receptor is weakly methylated, whereas in females reared by careless mothers it is strongly methylated (Figure 6). Thus, once they become adults, females reared by careful mothers express that receptor, and therefore detect the strong hormonal changes occurring at parturition, which triggers a cascade of processes that lead them to fall in love with their pups. In contrast, females bred by careless females do not express this gene due to the high methylation of the promoter. They therefore do not detect the hormonal changes at parturition, preventing them from falling in love with their pups. They merely continue to behave like females outside of parturition and thus neglect their young. Hence, in each generation females reconstruct the same level of parental behaviour as their mothers, leading to parent-offspring resemblance in parental behaviour.

Figure 6: The epigenetic transmission mechanism of maternal care behaviour in rodents. With this mechanism females reconstruct a level of parental investment similar to that of their mother[119]. The blue dots represent the positions of methyl radicals on cytosines.



Similar mechanisms are also found in the transmission of other traits such as stress responses, anxiety, cognitive abilities and reward responses, showing that the impact of early life effects is multidimensional. We have known for several decades that these early effects have the potential to affect development. It is now clear that some of these effects are also transmitted over at least several ten generations[120].

What would be the adaptive value of this type of heredity?

Fascinating as these studies are, they do not explain why evolution has favoured the transmission of such responses to the environment to future generations (Tinbergen's approaches 3 and 4). In other words, what is the evolutionary origin of such a form of heredity? How might passing on one's level of parental care to offspring have provided an evolutionary advantage? One might even think that low care to offspring might be strongly counter-selected, since it could only seriously diminish the survival of the offspring, leading to the extinction of the family lines.

This is a central issue to which we will return, but it appears that this transmission of maternal behaviour is only one facet of a behavioural syndrome generated by early life effects. These environmental effects are often referred to as stress, which conveys the idea that their effects are necessarily negative. In fact, since the first studies on the subject in the 1950s-1960s, it has been shown that these early life stresses also have beneficial effects, for example in the form of a better ability to cope with stress[121]. Therefore, in a stressful environment it can be advantageous to pass on a good ability to deal with stress to offspring.

On the other hand, we tend to think that there is always a single solution that works in all circumstances. For example, we all think that it is always best to provide as much parental care as possible, whatever the conditions. However, this is highly unlikely, and an excellent solution in one environment may be a poor response in another. In this case, early effects must be highly condition-dependent, and should be modulated according to the many parameters of the physical, biological or social environment. Differences in parental behaviour must therefore be seen as different strategies, each adapted to different environments. This implies that in order to understand their evolutionary origins, we need studies in a natural ecological context. For

example, it is conceivable that a mother stressed by the detection of predator urine odours may tend to neglect her offspring, which will then be better able to cope with that stress.

A phenomenon that also exists in humans

There is also considerable evidence in humans and primates for the transmission of maternal behaviour from mother to daughter. For example, for human child abuse, there is a striking transgenerational continuity as about 70% of abusive parents were themselves abused[122]. Similarly, mothers who were raised in institutions, and who therefore received little or no maternal care, show lower sensitivity towards their own children. Last, a mother's attachment to her own mother is a very good predictor of her daughters' attachment to her. All these processes raise societal problems when parental behaviour becomes inappropriate. As far as the underlying mechanisms are concerned, we are so used to thinking that the transmission of a trait implies *de facto* a sequencic determinism that we naturally tend to think that members of these families must transmit sequencic variants that lead them to behave in this way. However, the rodent case is not isolated and similar results have been obtained in other mammals including primates and humans[123]. Thus, as with the study of all physiological mechanisms, rodents[124] constitute an excellent research model for understanding the inheritance of parental behaviour in humans by making it possible to carry out research that would be impossible in humans, or even primates, in order to understand the determinism of this parent-offspring resemblance[125]. Therefore, this model is widely used in human psychology to study the determinism of such heritable variations of human parental behaviour.

Finally, it goes without saying that only a good understanding of these mechanisms can allow us to hope to define therapies able to break the vicious circle of this form of heredity of human mistreatment. Given what we know today about this type of heredity, keeping a purely sequencic vision of heredity would inevitably lead us into a *cul-de-sac* that would prevent us from defining therapies adapted to this particular type of heredity.

A new pathway for transmitting information across generations

If we return to the Diagram of information flows across generations as presented in **Erreur ! Source du renvoi introuvable.**, now we can add a new pathway of intergenerational transmission (**Figure 7**) involving an epigenetic first step in the daughters' brains, which leads them to reconstruct the same type of maternal behaviour as their own mother.

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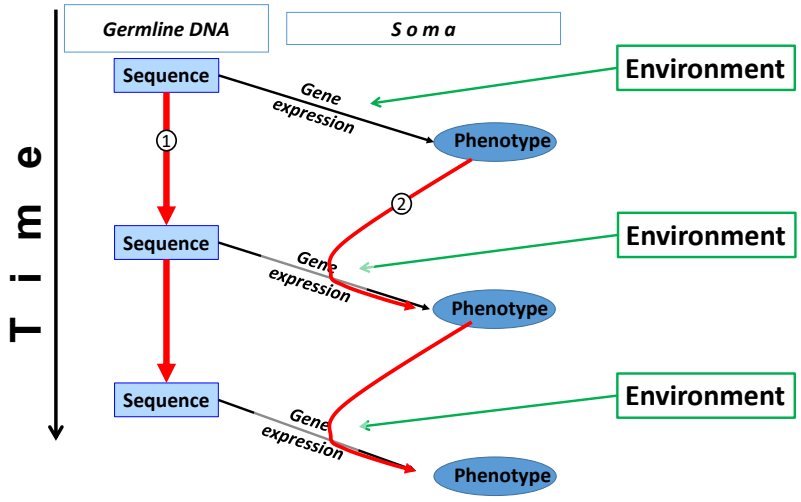
Figure 7: The intergenerational information pathway summarising the examples in this chapter. According to this pathway (arrow 2), mothers constitute for their daughters a component of their environment that affects their daughters' brain epigenetic state. These epigenetic states lead daughters to reconstruct their mother's behaviour. Arrow 1 represents the transmission pathway carried by the DNA sequence[126].



Yes, but...

You might object that even if you are now convinced of the reality of this inheritance mechanism, it might simply be an exception, albeit an interesting one, but that there is no need to give it more importance than it has. This is a thought that I am regularly given. However, we have seen that this type of transmission concerns many types of behaviour, and we will see in the following chapters that there are a whole series of other examples of this kind based on particularly sophisticated mechanisms.

Another common comment I hear is that I cannot talk of heredity in this case because nothing is really transmitted. The maternal behaviour of mothers is an important element of their daughters' environment which leads them to reconstruct the same behaviour as their mother, nothing more. And I am told that this is just an

effect of environmental variation and that it can be neglected. Without falling into a sterile semantic debate, I have been careful to define heredity as patterns of parent-offspring resemblance, because it is *the very existence of such parent-offspring resemblance that allows evolution by natural selection or drift (see Glossary)*. Whether or not something material is transmitted is therefore irrelevant. It could also be argued that information about the state of the environment is indeed passed on from mothers to daughters in the form of epigenetic marks. A third recurring objection is that this is not really inheritance because it does not pass through the gametes. However, the concept of heredity does not imply that parent-offspring resemblance must pass through gametes. A fourth objection is that experimental cross-fostering show how labile this transmission is and therefore such transmissions are unlikely to influence evolution. This argument is somewhat spurious insofar as such cross-fostering simply does not occur in nature, so that in nature this mechanism must effectively generate variation transmitted over multiple generations within family lines, variation that is therefore open to selection. Anyway, we will now look at other examples where these objections are not relevant.

Chapter 7
Hereditary effects of pollution

Anyone who has had the opportunity to observe beavers in their natural environment has had the opportunity to observe a phenomenon that is very important for their survival and reproduction. Beavers build dams on streams, which completely change the state of their immediate environment, and allow them to build a nest surrounded by water, with the entrance permanently underwater, thus removing the opportunity for many predators to interrupt their reproduction. Although it may seem harmless, this phenomenon has many consequences that go far beyond the survival of this species. For example, with climate change, beavers are able to survive further and further north in Alaska, where they are changing the environment in all the valleys, thereby accelerating the effects of global warming on the melting of permafrost.

The ability of species to change their environment is called "niche construction" and can strongly affect parent-offspring resemblance and the fate of natural populations. As a matter of fact, if organisms in one generation change their environment, all other things being equal, this implies that the selective and developmental pressures from the environment change over generations. This is particularly the case for organisms with low mobility such as plants and microorganisms. The phenomenon of niche construction must therefore strongly affect evolutionary dynamics and ignoring it would mean ignoring an entire evolutionary process.

In the case of the human species, this tendency to modify the environment reached a level probably unprecedented in the three and a half billion years since life first appeared on earth. We build houses, cities, roads and highways, bring water to our taps and fields, heat our homes in winter, cool them in summer, clear forests to grow crops, all of which change our environment and the selective pressures acting on us. If we go back in time, agriculture is an important and ancient form of niche construction that has significantly buffered seasonal variations in food resources. This practice was also responsible for the transition to a sedentary lifestyle and the human population boom that began about ten thousand years ago and that continues to this day.

In fact, all the ongoing ecological crises, from the local ones such as heavy metal pollution from mining activities or herbicide treatment of a private garden, to global effects such as the global biodiversity crisis, the increase of carbon dioxide in the atmosphere with its global warming consequences are all direct or indirect effects of our ability to build our ecological niche. There is of course a downside to this process, as all these environmental changes affect our health. In particular, we now live in a world containing many synthetic molecules which, because of their high persistence in the environment, accumulate year after year. These molecules even pass into the oceans or are carried by the wind over great distances, contaminating the entire planet.

In this chapter we will see how the effects of pollution by certain synthetic molecules widely used in agriculture not only negatively affect the biological functions of the polluted individuals, but also affect the health of their offspring over several generations, even if they themselves are never exposed to pollutants. In other words, some of the effects of pollutants on the body can be passed on to offspring over several generations even if the offspring develop in a pollution-free environment. Surprisingly and worryingly, these effects are therefore inherited!

It all started with a controversy

In 2005, *Michael Skinner*'s group at Washington State University in the United States of America published a paper in *Science*[127] that caused a lot of controversy because it reported results that seemed to be totally at odds with the dominant view of heredity according to the Modern Synthesis[128].

That article presented the effects on rat embryonic development of two molecules classically used in agriculture. These two molecules are endocrine disruptors in the sense that they mimic certain sex hormones and therefore strongly disrupt the development of embryos and in particular the development of their sexual organs. One is a fungicide classically used in the wine industry. It mimics oestrogens and their injection therefore mimics a supply of these female hormones. The other molecule is a pesticide classically used after the DDT ban that has an anti-androgenic function in that it blocks the effects of male sex hormones. In all of *Michael Skinner*'s experiments, each female in the experimental group was injected at the beginning of gestation with a low dose of only one of the two tested molecules. The authors had chosen to use these two

endocrine disruptors acting at different metabolic levels to demonstrate the generality of the effects of endocrine disruptors.

Surprising results

They found that the temporary exposure of pregnant rats to one of these molecules when their embryos' germ cells were differentiating induced greatly reduced sperm counts and motilities, associated with a significant increase in male infertility in their male offspring. No other significant morphological and functional effects on other organs were detected. This was a classic toxicological result: even at very low doses, certain molecules can alter the functioning of organisms, particularly if exposure to the pollutant occurs very early in life.

The surprise was that these strong toxicological effects were then transmitted to more than 90% of the male offspring of all the following generations, i.e. to F1 (offspring) down to F4 (great-great grand offspring) of the treated pregnant females (classically called F0). The general syndrome of the F4 was equivalent to that of the F1 and showed no sign of fading out. In other words, when administered to pregnant rats, molecules commonly used in agriculture can affect the development of their embryos, leading the male offspring to have serious reproductive deficiencies that can even lead to total infertility in 8% of cases over at least 4 generations, and this when none of the offspring was ever in contact with the endocrine disruptors in question.

In 2005, cases where environmental effects affect first generation offspring were beginning to be reported, but this was the first time that such effects were shown to be more than just simultaneous exposure to a pollutant, as they were passed on to offspring over several generations. According to the definition of heredity set out in Erreur ! Source du renvoi introuvable., this is definitely an inheritance process. Thus, following an environmental stress, the young F1 males had acquired a new phenotype (a reproductive dysfunction), and then passed it on to their male descendants until at least the F4 generation.

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Everything pointed to an epigenetic process

That study not only described a surprising case of heredity, but also provided a series of arguments suggesting that this transgenerational transmission is based on epigenetic mechanisms:

- 25 areas of the genome showed aberrant methylation patterns in the germline in association with reduced fertility relative to controls;
- The high transmission rate of the reproductive impairment (more than 90% of males inherited it) makes it highly unlikely that this parent-offspring resemblance is due to a mutational effect of pollutants;
- The reproductive impairment observed in the offspring was very homogeneous in all treated lines, which does not argue for mutational effects, as mutations are supposed to be random relative to the environment, and thus should generate some variation in response across generations. Conversely, epigenetics can produce a homogeneous response across family lines because epigenetic changes are highly targeted to very specific parts of the genome leading to a homogeneous response to the same stress;
- Last, the period of exposure to the pollutant corresponded to the period of germline differentiation of the embryos, i.e. the period during which the methylation programming of the germline takes place.

All these arguments ruled out a purely sequencic explanation and pointed to an epigenetic process. However, being partly correlative, these interesting results did not allow to definitively claim that the observed epigenetic changes are actually the cause of the fertility changes, nor of the transgenerational transmission. It can therefore be said that at that stage the case against a mutational interpretation was strong but not conclusive.

A classic backlash

Of course, such results seemed to contradict several of the principles of the Modern Synthesis developed in Erreur ! Source du renvoi introuvable., so they triggered a series of reactions challenging their reliability[129]. Moreover, as this study involved molecules synthesised and marketed by multinational firms, it could affect the future of these firms, and one can imagine that they would seek to discredit such studies. This is the typical reaction to that disturb large international trusts[130]. I have seen colleagues confronted with this kind of behaviour during my career.

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Facts are stubborn

Nevertheless, the facts are stubborn, and *Michael Skinner*'s team had taken many experimental precautions to avoid all possible pitfalls. For example, the experiment included a control group of pregnant females injected with no pollutant, and these control family lines never showed any sign of dysfunctional male reproductive function. Similarly, the authors showed that the male progeny of crosses of second generation (F2) males with females whose ancestry had not been treated showed the same reproductive deficiencies. Conversely, crosses of females whose grandmother had been treated with males whose ancestry had not been treated produced normal males, confirming that the information is passed on through the sperm and not the egg.

Furthermore, a series of subsequent studies showed that in the great-grand offspring (F3) of treated (F0) females, more than 400 genes were expressed differently than in control rats[131]. Finally, when F3 females, whether or not they were offspring of treated pregnant great-grandmothers, were given a choice between a male whose great-grandmother had not been treated versus a male whose great-grandmother had been treated, the females showed a clear preference for the males whose ancestry had not been treated[132]. This showed that females are able to detect the difference between males with and without a history of pollution and that treatment can affect the fitness of male offspring across generations and thus change the evolutionary fate of the family lines.

It could be argued that these results are a relatively marginal exception as I have so often been told. However, the field of toxicology has produced similar results with other types of pollutants[133]. DDT, paraffin, plastic additives such as bisphenol A and dioxin can each trigger transgenerational health effects in rats, such as obesity and ovarian disease, each of which is associated with different epigenetic changes in the gametes of the affected individuals[134]. All of this strongly suggests that *Michael Skinner*'s team's research is not a rare outlier.

Another argument in favour of the generality of such transgenerational effects of pollutants is that these results in rodents are reminiscent of the current sharp loss of human male fertility[135]. Could such declining male fertility be due to the pollution we are blithely accumulating in our environment? Should we not take precautions against this possibility? For example, shouldn't we think more about the minimum distance from houses or schools at which the spreading of these molecules potentially responsible for the fall in human fertility is allowed? These are all important questions raised by this type of result.

Skinner’s work has had the merit of strongly suggesting the possibility that environmental factors (such as endocrine disruptors) can reprogram the germline generating transgenerational abnormalities, with all the obvious implications for evolutionary and medical biology.

Chapter 8
Heritable consequences of aversive conditioning

A few days ago, after a storm, I went for a walk on the cliffs of *Kermaden* in *Cap Sizun* (south Brittany) where I am writing these lines. On the moor I came across a brood of wrens (*Troglodytes troglodytes*) that had just come out of the nest. At first the parents didn't see me and I was able to get close to the chicks that had just left their nest. I could even touch the back of one of them. Then the parents saw me and started to alarm, coming as close as possible to their young without exposing themselves to the danger I represented. They were visibly panicked. In fact, this panic on the part of the parents is the best indication to the chicks that there is danger. It is certain that if I had then sought to approach these same chicks again, they would have run away from me immediately having learned of their parents' panic. This is a very powerful social learning mechanism that has been known for a long time, but until very recently we did not know much about the cognitive mechanisms involved.

Similarly, we all know people who have a panic fear of completely harmless things. For example, the fear of spiders or slugs, most of which are completely harmless. Very often we will find that one of the parents had a fear of spiders or slugs and somehow passed it on to their children. And actually, in humans as in animals, the best way to teach young people to be wary of something is to panic about it yourself.

This is why animals (including us) can easily be conditioned to be afraid of something quite benign, simply by systematically associating that benign thing with something unpleasant or dangerous. This is a well-known mechanism, but again, until recently the proximate mechanisms involved were unknown. This was the goal of the study by *Dias* and *Ressler*[136] mentioned in the introduction to this book to study the proximate mechanisms of this learning[137]. But above all, that article showed that, quite unexpectedly, this aversive conditioning has transgenerational effects! How is this possible? This is what we will see in this chapter before developing other examples in the following chapters.

The inheritance of aversive conditioning

Remember those mice with increased sensitivity to a particular smell for the sole reason that their parents or grandparents (with whom they had never been in contact) had been conditioned to be afraid of that smell[138]! We wondered how such a phenomenon could occur, how offspring could inherit traits acquired by their ancestors during their lives, without ever having met them. As the authors had taken every precaution to avoid any social influence by the parents (*in vitro* fertilisation, cross-fostering with implantation in the uterus of another female etc.), these experiments showed that the effects of traumatic experiences are inherited through other means than social influences, and that the only way to transmit them is through gametes. This article provoked reactions because it raised a series of questions that at the time, in 2014, seemed plain esoteric. We will see that since then, ideas have evolved considerably in this field.

More generally, given the rapidity of current and past environmental change, it is likely that the many adaptive responses observed in nature belong to phenotypic plasticity and not to a mutation-selection process. Such adaptive responses to environmental stimuli often involve alterations in the structure and function of the nervous system.

Beyond these mechanisms of accommodation unfolding over an organism's life, we will see that *Dias* and *Ressler* study documents the existence of the inheritance of environmental effects without involving genetic modification. As we have seen, such inheritance pathways are expected as they can provide significant selective advantages by allowing one generation to shape offspring according to the foreseeable conditions by acting on the way offspring use their genetic information. The advantage of such inheritance systems would clearly be in their rapidity, as they would involve *changes in gene expression rather than mutations.* You will note from what we saw in **However,** before going into the description of these many striking examples, it is necessary to take the time to introduce a fascinating and rapidly growing field of organismal biology, that of epigenetics.

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that all these considerations point to an epigenetic nature of such mechanisms. And indeed, these mechanisms turn out to be essentially epigenetic in nature

The principles of the mouse experiment

An important element is that as rodents often live in dark habitats and are most active at night, evolution has fostered a particularly sophisticated sense of smell in these animals allowing them to detect the presence of predators.

The concerned experiment involved the pre-breeding conditioning of mice by systematically associating a benign but detectable odour, that of acetophenone, with the stress of an electric shock. There was also a control with a group of individuals who were made to smell another odour, propanol, without associating it with an electric shock. Authors then assessed the effects of such conditioning with the "odour-potentiated startle" test that measured the increase in amplitude of a simple reflex to a stressful noise depending on whether the conditional stimulus (in this case, odour) was present or absent. Mice freeze whether the odour is present or not, but this reaction is much stronger and longer lasting in the presence of the odour. The odour-potentiated fear test thus reveals an increased fear response to the stressful noise in the presence of the odour. The study also had two other experimental treatments, identical but where propanol was associated with danger and acetophenone was benign.

After a series of such coincidences, whenever they smelled acetophenone, conditioned but not unconditioned mice overreacted to a stressful noise, revealing an enhanced fear context, which then persisted throughout their life.

The transgenerational effect

Unexpectedly, not only did mice conditioned to fear acetophenone subsequently show increased sensitivity to acetophenone, but their unconditioned first- and second-generation offspring also showed increased sensitivity to acetophenone (but not to propanol), and *vice versa* in the experiment swapping the roles of acetophenone and propanol. The offspring were therefore over-sensitive to the smell for which their parent or grandparent had been conditioned.

In another experiment, by varying the odour concentration, an aversion test showed that the offspring of individuals conditioned to fear acetophenone (or propanol) detected acetophenone (or propanol) at lower concentrations than the offspring of unconditioned mice. Of course, all this was achieved when the offspring had never been exposed to any of these odours prior to testing and in the absence of contact with their parents. These results suggested the transmission of a higher state of expression of the gene coding for the receptor to the molecule used for aversive conditioning (a gene called *Olfr151* and *Olfr6* for acetophenone and propanol respectively)[139].

Mechanisms involved

As we saw in **Erreur ! Source du renvoi introuvable.**, demonstrating that a parent-offspring resemblance persists over at least two generations through the male pathway[140] provides evidence for real transgenerational processes. The behavioural results are therefore remarkable in themselves. But the main surprise was found in the study of the mechanisms of this parent-offspring resemblance, which involved the use of several complementary approaches.

Neuroanatomical effects

Aversive conditioning of fathers to an odour causes an increase in the number of odour-specific olfactory neurons (acetophenone or propanol) in the father, but also in his first and second generation offspring (obtained by *in vitro* fertilisation to eliminate any social transmission). This suggests that this observed increase in the number of olfactory neurons is responsible for the offspring over-sensitivity to the aversive odour. It would therefore seem that aversive conditioning not only affects the neuroanatomy of the conditioned individual, but also that of his or her descendants over at least two generations.

Cross-fostering shows germline inheritance

The study also included a cross-fostering experiment to eliminate any possibility of non-gametic transmission. This experiment differed from the one described above as it involved aversive conditioning of females to fear an odour (acetophenone or propanol). This tested the transmission via female gametes while allowing for cross-fostering. Again, the F1 showed the same behaviour and neuro-anatomical changes, whether they were raised by their biological (conditioned) or adoptive (unconditioned) mother, comforting the conclusion that this inheritance effectively occurs via the male or female germline pathway.

The genetics of olfaction

To go farther, the authors used their knowledge of the neuro-genetics of olfaction in mice. In particular, as in most mammals, in rodents the receptor neurons of the main olfactory epithelium in the brain have a projection into the nasal epithelium. Each of these neurons expresses one (or more) of the genes of the olfactory receptor family[141]. Rodents are unique in that they have a large number of such olfactory receptor genes, and thus have a wide variety of neurons specialised in the detection of one (or more) specific odorant molecule(s). They therefore have a sense of smell that would make the best perfumery noses green with envy. These neurons are well mapped in the main olfactory epithelium of the brain, and the authors chose acetophenone and propanol because their receptor genes (*Olfr151* for acetophenone and *Olfr6* for propanol) are never expressed in the same neurons, and their sensory pathways are therefore clearly independent.

Fear conditioning affects the methylation of specific genes in gametes

They then found that the promoter of the gene involved in the detection of the aversive odour (and not that of the other odour) was less methylated in the sperm of conditioned than in unconditioned males. This demonstrated the surprisingly specific targeting of epigenetic changes; only the gene involved in detecting the aversive odour was affected. Furthermore, this methylation state was found in the gametes of both offspring and grand offspring. These epigenetic marks seem to escape the waves of demethylation-remethylation that occur at different stages of reproduction (see **However,** before going into the description of these many striking examples, it is necessary to take the time to introduce a fascinating and rapidly growing field of organismal biology, that of epigenetics.
)[142].

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No detectable methylation change in the brain

Dias and *Ressler* naturally looked for an epigenetic change in the brains of conditioned mice. Indeed, an intuitive way to generate hypersensitivity to acetophenone would be simply to overexpress the receptor gene involved in the detection of that odour (*Olfr151* for acetophenone and *Olfr6* for propanol) in the cells that express this gene and are involved in the detection of this molecule. Such overexpression would imply a demethylation of the promoter of the concerned gene and would lead these neurons to be more sensitive to that specific odour. However, no methylation changes were detected in the brain, neither in the conditioned mice nor in their offspring. It should be noted, however, that there may be several explanations for this result. In particular, as we have seen, DNA methylation is not the only mechanism involved in the regulation of gene expression

Remark

Very often these results are presented by talking about the heredity of fear. However, it should be noted that this formulation is somewhat misleading. In these experiments, the conditioned mice do not freeze when they smell the concern odour. They are only more likely to be frightened if something surprises them. Similarly, the mice do not inherit their parent's fear (they do not freeze when they smell that odour). They inherit the heightened sensitivity to the odour that their parents (grandparents) have been conditioned to. Thus, this transmission leads to offspring being more sensitive to danger signs rather than more fearful in general.

This process is reversible

In a later study, the same research team showed that it is possible to interrupt this transmission by regularly presenting the smell alone without associating it with any danger[143]. This study replicated and generalised the previous results. Replication is important for any research because it eliminates the possibility that something special and uncontrolled in the first experiment may have generated the results. The new study used the same conditioning protocol as before but added an experimental group of mice that after being conditioned to associate an odour to danger, were given a long series of presentations of the same odour but this time not associated with an electric shock in order to study the extinction of the previous conditioning.

They found that after the extinction treatment, the group of mice subjected to the extinction protocol did not differ from an experimental control group that only smelled the odour without association with the electric shock. Thus, the fear conditioning can be reversed by regularly presenting the same stimulus in the absence of danger.

This extinction property is important for two reasons. First, it suggests an important avenue for treating people with paralyzing fear for trivial things. There are actually therapies that practice this type of protocol globally. On the other hand, this study highlights a major property of non-genetic inheritance. While genetic inheritance involves almost irreversible processes due to the high fidelity of DNA duplication, *non-genetic inheritance is reversible*. We will return to this important feature in the third part of this book. At this point let us just say that the Modern Synthesis considers this reversibility to be a weakness of non-genetic inheritance,

which disqualifies it as an actor of evolution. On the contrary, rather than claiming that low fidelity does not allow for very long term transmission, we should emphasise the fact that it brings reversibility, which constitutes a real strength of non-genetic inheritance. More to follow.

The many enigmas raise by all these studies

In summary, it is clear that fear-conditioning mice changes their neuroanatomy, as well as the epigenetic status of relevant genes in the gametes of conditioned individuals. How can this be possible? This seems to be contrary to everything we have learned, as the germline is supposed to be immune to all environmental influences. Furthermore, these epigenetic changes in the gametes seem to escape the waves of demethylation-remethylation during reproduction and, also, seem to direct the development of the offspring in such a way that they inherit an increased sensitivity to the odour associated with a danger.

These results raise a range of equally surprising questions, which are addressed by *Moshe Szyf* in his commentary. Here is a "Prévert-style inventory" in the order in which the mechanisms should occur in real life.

- 1- How to explain the absence of any detectable epigenetic change in the parts of the brain involved in the detection of the odour in question after fear conditioning? We have just sketched out a possible answer above, but this needs to be studied in detail.
- 2- How are both the memory of the smell and its association with a traumatic experience transmitted?
- 3- By what mechanism can conditioning affect the epigenetic state of very specific genes in gametes? This goes against the very logic of the so-called Weismann barrier. In other words, are there communication pathways between the environment and specific genetic loci in gametes?
- 4- How can epigenetic states be maintained through the waves of demethylation-remethylation that occur during reproduction?
- 5- How can such simple epigenetic states of the gametes affect the development of the offspring's brain in such a way as to reconstruct the specific sensitivity developed during the conditioning of recent ancestors?[144]
- 6- What selective advantage is provided by such non-genetic inheritance processes?
- 7- A central question in assessing the potential impact of such a form of inheritance is for how many generations can this transmission persist?
- 8- Finally, on an evolutionary scale, at the end of his commentary, *Moshe Szyf* asks a fundamental question to which we will return in **Chapter 10**: is there a mechanism that would fix such epigenetic changes in such a way as to inscribe their effects in the DNA sequence itself, thus affecting the course of evolution? This is indeed a major question.

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Therefore, there would be several ways of encoding heritable information!

Already in the preface to this book, and then in this chapter, we saw how much *Dias* and *Ressler* study raised all the major questions that we have just listed. But, more insidiously, this major result also went against a widely accepted idea according to which when the environment changes too often (i.e. every few generations), evolution, instead of favouring the engraving of the concerned information into the genetic sequence, should favour learning from the environment. That view proposed the existence of two possible strategies, one by irreversible encoding into the DNA sequence and the other eminently reversible by learning from the environment. However, what *Dias* and *Ressler* and many others were showing is that in fact there is a series of mechanisms that lie between these two extremes and allow a wide range of adaptations to be transmitted with varying levels of reversibility. Another important discovery is that within this range of processes, epigenetics occupies a central place by offering intermediate levels of reversibility. It is this major originality that gives epigenetics its importance in evolutionary processes. This was a major discovery which, as we shall see in the third part, is in fact at the very heart of this book.

Again, as scepticism knows no bounds, one might persist in asking whether this is a very special, or even unique, case. We will see in the next chapter that it is far from being a unique case, and we will start to answer question 3 in particular. Finally, we will address above questions 2, 3, 4, 7 and 8 partially but in an integrative way in the third part of this book.

Chapter 9
Heredity of acquired parental phenotypes in response to environmental factors

Eating too much sugar or fat in combination with a lack of exercise can lead to various metabolic diseases, including obesity and type II (i.e. acquired) diabetes. Similarly, dependence on alcohol (or other drugs) affects the functioning of the body and can lead to serious diseases such as liver cirrhosis, often resulting in death. These are well-documented facts that on a societal scale generate serious and very expensive public health issues, some of which are increasing so rapidly in Western countries that they are often referred to as epidemics, which is a nice oxymoron since these diseases are not produced by contagious infectious agents. But would you imagine that the serious consequences of such poor lifestyle habits can then be passed on to offspring, even if they adopt a healthy lifestyle? How could this be possible? What would be the mechanisms responsible for such transmission? This is what we will discuss in this chapter.

Acquired diabetes

In September 2015, I was invited to a seminar in Falmouth, Cornwall, which brought together evolutionary and medical researchers, particularly specialists in metabolic diseases such as obesity and diabetes. The theme that united us was the importance of early in life effects. It was a very rewarding experience that led to a special issue of the *Philosophical Transaction of the Royal Society of London*, to which I contributed an article, and to which we will return in **Chapter 16** and **Chapter 17**. I hence discovered a whole field of research on so-called non-communicable diseases, which do not result from an infectious agent (virus, bacteria, etc.), but which are nonetheless the source of numerous disorders, sometimes disabling, even fatal. One of these is diabetes.

The discussions I had with attending physicians opened my eyes to a new world. I kept in touch with *Mark Hanson*, Professor at the University of Southampton in the UK. He told me about obesity and its associated metabolic disorders such as diabetes. It is estimated that around one billion people worldwide are overweight[145], and thus susceptible to develop type II diabetes, which has two components, glucose intolerance and insulin resistance, the pancreatic hormone that regulates the blood glucose levels.

It made me realise how urgent it is to develop therapies to curb this silent epidemic. *Mark Hanson* told me that when interacting with politicians, they were regularly confronted with a reaction that makes perfect sense for a politician whose role is to make informed decisions: "Okay. What should we do?" and that was the end of the discussion because, in fact, not much was known at the time (not so long ago) about the origins of this non-communicable disease and even less about its transmission. The most surprising thing is that once developed by young men, this disease is then passed on to their male descendants. Men who have acquired type II diabetes because of their poor lifestyle (reduced physical effort) and unbalanced diet (too much fat and sugar, constant eating) produce offspring who themselves become obese and diabetic regardless of their lifestyle. In other words, surprisingly enough, diabetes can result from the father's inadequate diet[146]. We will see that this parent-offspring resemblance results from the transmission of information by the sperm cells of fathers who became obese before reproducing. This implies that the father's diet and lifestyle can affect his own sperm in such a way that their male offspring develop the same syndrome as their father.

Once again, how is such parent-offspring resemblance possible? What are the molecular mechanisms involved, and how is the associated information carried in the sperm? These are all questions raised by this new form of inheritance of traits developed during life in interaction with the environment

Acquired diabetes is inherited through sperm cells

A few months later, in March 2016, the journal *Science* published two papers in the same week, which I rushed to send to *Mark Hanson* because they both shed new light on the inheritance of diabetes and opened up an incredible avenue of research[147].

The first article, co-authored by Chinese teams[148], demonstrated in mice that the transmission of diabetes following a poor lifestyle does indeed pass through the sperm. They fed mice a diet that was either very high in fat (60% fat) or healthy (10% fat) for 6 months from the age of 5 weeks, i.e. early in their lives. The mice

fed the high-fat diet became obese and insulin resistant and glucose intolerant, and thus had type II diabetes, whereas the mice fed the healthy diet did not develop this metabolic syndrome. The researchers then injected the head of a diabetic male sperm into the egg of a female fed a healthy diet. The *in vitro* fertilised eggs were then implanted into the womb of another female fed a healthy diet. Despite being fed only a healthy diet, the resulting young males were not obese but developed diabetes as revealed by levels of glucose intolerance and insulin resistance very similar to the diabetic syndrome in their father. Thus, although fed a healthy diet, they developed severe diabetes.

The conclusion was that information had been transferred from the fathers to their offspring and that that information was entirely contained in the sperm cells of diabetic males.

A story of microRNA (see Box 2)

The study also determined the exact nature of the molecular carrier of this sperm-borne information. There were several indications that this information could be carried by RNA in the sperm (see **However,** before going into the description of these many striking examples, it is necessary to take the time to introduce a fascinating and rapidly growing field of organismal biology, that of epigenetics.
). Firstly, sperm cells have a wide variety of RNAs and in particular micro RNAs, i.e. RNAs less than 200 nucleotides in length. Second, the literature had already reported examples of transmission via the sperm pathway by small RNAs contained in sperm cells[149]. Third, many of these micro RNAs do not carry a coding sequence and are in fact fragments of ribosomal RNA (rRNA) or transfer RNA (tRNA)[150]. And finally, because of their sequencic structure, RNA, like DNA, are particularly well suited to carrying a wide variety of information.

The authors, therefore, extracted all the RNAs of the sperm cells of males fed fat or a healthy diet, and injected these extracts into eggs resulting from the fertilisation of an egg and a sperm from individuals fed a healthy diet. They found that when they injected total RNA extracts from sperm cells of diabetic males (and not from healthy males) the resulting male offspring developed glucose intolerance similar to that of the males from which the RNA extracts were obtained, but did not develop the insulin resistance of their father.

Thus, in this study, total RNA extracts from sperm cells of diabetic males carried all the information necessary for the induction of glucose intolerance, but not that for the induction of insulin resistance. This suggested that the information present in sperm cells regarding this second symptom is carried by other types of avatars such as DNA methylation or histone modifications[151]. As total RNA extracts from sperm cells contain a wide variety of RNAs in terms of sequence and size, the authors then compared the diversity of RNAs in the sperm of high-fat-fed males with that of healthy-fed males. They found that in addition to the already known RNAs in mature mouse sperm, RNAs from sperm from healthy and overfed males differed mainly in the 30-34 nucleotide tsRNAs. They then injected tsRNAs extracts of 30-34 nucleotidic from diabetic males into a normal egg and found that this was sufficient to transmit glucose intolerance, showing that these 30-34 nucleotidic tsRNAs in the sperm are the avatars of the information necessary for the development of glucose intolerance.

It should be noted, however, that in another study where diabetes was generated by a diet enriched in both fat and sugar, injecting sperm RNA extracts is sufficient to transmit both glucose intolerance and insulin resistance[152]. This result is consistent with the fact that a diet enriched in both fat and sugar leads to much more severe forms of diabetes than a diet enriched only in fat. This second study also shows that such environmentally induced phenotypic changes persist over several generations. Hence, these small RNAs carry very precise information about the environment, and this with equally precise effects on the phenotype of the offspring.

It was shown subsequently that the tsRNAs responsible for diabetes transmission are in fact altered because the absence of the gene encoding for the enzyme that adds protective methyl radicals to tRNAs alters the profiles of sperm tsRNAs and abolishes the transmission of glucose intolerance mediated by tsRNAs[153].

Heredity of the effects of nutritional deficiencies

Beyond their thoroughness and surprising nature, these results raise the question of the origin of these small RNAs (see **Box 2**) present in the sperm of mice made diabetic by a diet enriched in fat (or fat and sugar). This is precisely the question studied in the second article published in *Science* in the same week of March 2016[154]. That second article deals with the transmission of another metabolic disorder acquired by protein-deficient parents (a common disorder in vegetarians), which is then transmitted to offspring in that they show the same dysfunction of hepatic cholesterol synthesis as their parents. So this is another example of parents under environmental stress acquiring a metabolic disorder and then passing it on through the male germline, showing that the transmission of diabetes is far from unique.

The origin of sperm RNA (see Box 2)

This second study also focuses on male inheritance through the germline. It uses *in vitro* fertilisation to show that dietary protein deficiency in the father can affect the metabolism of his offspring via information localised in sperm cells. Sperm cells from protein-deficient males have two to three times more small transfer RNA derivatives (tsRNAs) of size between 28 and 34 nucleotides in size than sperm from non-deficient males. On the other hand, since sperm leaving the testis have very few tsRNAs, the question arises as to the origin of the tsRNAs found in mature sperm cells. After leaving the testes, sperm mature for several days in the epididymis, an organ just outside the testes. In fact, sperm cells acquire their tsRNAs while in the epididymis. The epididymis lumen contains many epididymosomes, tiny vesicles surrounded by a cell membrane, which by fusing with the sperm membrane inject their contents into the sperm cells. Analysis of the contents of the epididymosomes showed that they indeed contain tsRNAs, similar to those found in mature sperm cells. Furthermore, by incubating sperm cells collected at the testis exit (and therefore devoid of tsRNAs) with epididymosomes, the authors were able to reproduce *in vitro* this transfer of tsRNAs suspected of taking place in the epididymis, because at the end of this incubation, the sperm cells did contain the tsRNAs that were initially in the epididymosomes. Finally, other studies show that in maturing sperm cells the proportion of tsRNAs among the small RNAs increases strongly during maturation in the epididymis[155].

Thus, this second study confirms and reinforces the first in the context of the transmission of another metabolic disorder, showing that the lack of dietary protein in the father can (*i*) influence his metabolism, (*ii*) affect the level of tsRNAs along the reproductive tract and hence in the sperm, and (*iii*) these same tsRNAs can regulate the expression of certain genes during the development of the offspring, leading them to reconstruct the same metabolic disorder as their father.

To sum up

Together, these two studies show the reality of the transmission of metabolic disorders acquired before reproduction. The first study shows that individuals who acquire diabetes before reproduction subsequently transmit this disorder to their offspring through a pathway that involves small derivatives of transfer RNAs carried by sperm cells. The second study confirms this type of non-genetic inheritance pathway for another metabolic disorder and strongly suggest that the concerned tsRNAs are of somatic origin, as they are injected into sperm cells during their passage through the epididymis[156]. These beautiful results shed new light on non-genetic inheritance, and open promising avenues for treating these metabolic disorders and interrupting the intergenerational cascade generated by such transmission.

It should be noted, however, that these studies focus mainly (but not exclusively) on transmission via male gametes for purely technical reasons[157]. In general, it is quite possible that this type of RNA transmission also exists via the female germline[158].

A very common phenomenon

These cases are not rare

One might wonder how general these phenomena are. However, there is now a long series of documented cases of transmission of acquired phenotypes by parents (summarised in **Box 3**). Examples include the effects of parental diet and associated metabolic disorders (this chapter), various mental stresses (anxiety, or parental behaviour as discussed in Erreur ! Source du renvoi introuvable., **Chapter 8**), exposure to chemicals (**Chapter 7**), but also the effect of exercise, or alcoholism, in mammals and a wide range of living organisms (**Box 3**). It can therefore be said that "*Increasing evidence now suggests that sperm small non-coding RNAs (sncRNAs) can mediate intergenerational transmission of paternally acquired phenotypes, including mental stress and metabolic disorders*"[159].

The recurrence of such observation made on various types of stress (**Box 3**) led to the proposal of the concept of "*spermatic RNA code*" to sanction the fact that sperm cells carry an RNA signature specific to each type of stress participating to parent-offspring resemblance[160]. This shows that the role of the RNA molecule and its numerous derivatives as a potential vector of heritable information has been largely overlooked for a long time. It is now necessary to consider RNAs as part of the information transferred by gametes alongside their DNA. RNA and its modifications (some of which involve interactions with microbes living in or on multicellular organisms) must today be considered as one of the major molecules of heredity. We will return to this subject in **Chapter 13** to **Chapter 16**.

The intergenerational stability of such effects

It could also be argued that in some of the cases discussed above transmission is only documented between F0 individuals who have been environmentally stressed (such as excess fat, or protein deficiency) and their direct descendants (F1). However, the transfer of small transfer RNA derivatives to the sperm is indeed a transfer of

information from parents to offspring, leading to parent-offspring resemblance. Thus, in these cases, parent-offspring resemblance does not result from a simultaneous exposure process, but at least in part from actual intergenerational processes.

Box 3: The ubiquity of the inheritance of initially plastic effects of environmental stresses

In addition to the many examples developed or cited in Erreur ! Source du renvoi introuvable. to Chapter 9, the heritability of environmental effects has been demonstrated for a wide variety of stresses and species[161]. The list would be too long to be exhaustive here, but there are excellent review articles on the subject. These intergenerational effects concern a wide variety of traits such as:

- Mental stresses such as anxiety and depression[162] or addiction to various molecules as alcohol or various drogues[163];
- Diet impact on basic cell metabolism[164];
- Various behavioural traits such as parental behaviour (Erreur ! Source du renvoi introuvable.);
- The effects of exposure to various pollutants (**Chapter 7**);
- Delays or interruptions in development;
- Infertility, and various metabolic diseases, such as obesity, starvation effects and diabetes[165].

As for the taxonomic spectrum of this kind of inheritance, these studies concern nematodes (see examples in *Caenorhabditis elegans* below in this box), insects[166], fish, rodents and many examples in humans[167]. There is also a whole body of literature on these subjects in plants and microorganisms[168]. As far as plants are concerned, following a conference in Marseille, one of the people in the audience told me afterwards that he had not learned much from my talk because he had known for a long time that plants can transmit traits that they have developed in response to the environment. And he was right, and there are several reasons why this type of non-genetic inheritance is probably more common in plants than in animals.

Beyond humans, one of the organisms for which there is a lot of information on the transmission of the effects of environmental stresses is the nematode worm *C. elegans*, where various effects have been shown to be transmitted over many generations, i.e. at least 3 generations[169], 14 generations[170], 20 generations[171], 25 generations[172], 40 generations[173] and even 80 generations[174] depending on the study. In all these studies, the number of generations reported did not correspond to the number of generations for the effect to disappear, but rather to the number of generations during which the experiment was carried out. It is therefore a minimum duration. For example, in the study that covered 80 generations, it should read "at least 80 generations" because, apart from the fact that the authors had the merit of testing the persistence of environmental effects over so many generations, they stopped there not because the effect had disappeared but because they had to publish this remarkable result[175].

In addition, a recent study adds to this very long list of examples of non-genetic transmission of environmental effects the case of cold resistance in humans and mice[176]. In the latter, exposure of males but not females to cold before pairing leads to male offspring with a metabolism that better protects them from diet-induced obesity. This modification is associated with methylation changes in the fathers' sperm. Since mice reproduce faster than seasonal changes, this phenomenon could be an adaptation to seasonal changes. In humans, it could be an adaptation to different climates and would explain the large differences in sensitivity to cold temperatures between individuals depending on where they grew up.

Furthermore, it appears that in mammals the transmission of small non-coding RNAs is more frequent through the male than the female pathway. This difference may be due to the fact that in this taxon females, but not males, can readily affect the phenotype of their offspring during gestation and lactation. Globally, however, there is no reason to believe that the overall capacity to transmit the adaptations acquired by recent ancestors differs between the sexes. It is likely that only the pathways change, depending on the constraints of each sex, among other things.

Moreover, in most examples transmission has been demonstrated well beyond the second generation. One of the most striking cases is that of the nematode worm *Caenorhabditis elegans* (called *C. elegans*), where the transmission of epigenetic states has been shown to persist for up to at least 80 generations without showing any sign of fading out (see **Box 3**)! If this could be transposed to humans, counting 25 years per generation, 80 generations would represent 2,000 years. Thus, an environmental factor that affected an ancestor at the time of Christ could have modified the expression of genes and thus the physical, physiological or behavioural characteristics of the ancestor at that time, and in such a lasting way that these effects would still be inherited today! Even if one can discuss the relevance of such an extrapolation, it is clear that such very long-term effects are integrated into our population estimates of trait heritability, which we nevertheless always interpret in sequencic terms. This does give pause for thought as to what we mean by heredity and how it cannot be reduced to the transmission of the DNA sequence alone.

It should be noted here that small non-coding RNAs play a crucial role in the first generation (from the environmentally stressed F0 to the F1). This role is then probably transferred to the establishment of different epigenetic states in the F1 affecting the phenotype and the germline, and which then ensure persistence from F1 to F*n*, *n* being an unknown number because I know of no study that has persisted beyond 80 generations, which is already enormous. One can imagine also that the accumulation of the same environmental stress over several generations can reinforce the transmission fidelity of the phenotypic response to the environment in question from generation to generation.

Conclusion

Soma to germline communication

An important aspect of all the above examples of non-genetic inheritance is that they show that somatic cells can, under the direct effect of the environment, transfer small non-coding RNAs (see **Box 2**) into maturing gametes, thereby altering gene expression and thus offspring phenotype, leading to parent-offspring resemblance. As a result, somatic cells would modify the information carried by gametes, potentially leading to a persistent parent-offspring resemblance over one or even many generations[177]. In view of the accumulating molecular evidence for such soma to germline communication, Chen and colleagues even speak in their review of the subject in *Nature Reviews, Endocrinology*, of the "… *the flow of information from the environment to somatic cells and then to sperm*"[178].

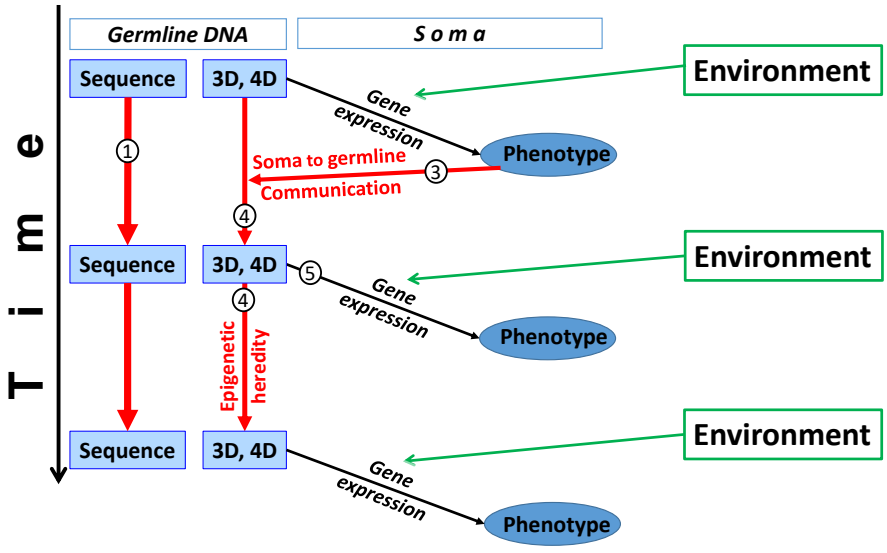
Major consequences in medicine and evolutionary biology

In another review article published in *Nature Reviews, Endocrinology* in June 2019, North American, Chinese, French and German authors analyse the potential impact in medicine of the existence of intergenerational or transgenerational epigenetic effects through the male or female germline pathway[179]. They claim that these recent findings have "*the potential to revolutionize our understanding of the aetiology of many human diseases that originate from environmental factors*". It is the purpose of this book to argue that this conclusion can be broadly extended to the whole of biology and especially to evolutionary biology, which is centrally concerned with parent-offspring resemblance.

A new pathway for transmitting information across generations

If we now return to the diagram of information flows across generations as presented in **Erreur ! Source du renvoi introuvable.**, we can add a new pathway of intergenerational transmission (**Figure 8**).

Figure 8: The intergenerational information transmission pathway summarising the examples in Chapter 7 to Chapter 9. This pathway has a first step (arrow 3) involving soma to germline communication, and a second step (arrow 4) occurring in the next generation and relying on the intergenerational transmission of germline epigenetic states that will then affect the phenotype of subsequent generations. A third step (arrow 5) is that epigenetic state in the gametes then affect the development and thus offspring phenotype. While arrow 3 is known to involve mainly small non-coding RNAs in the gametes, arrow 4 probably involves mainly epigenetic marks that persist across generations. Note that, compared to the previous diagrams, the germline now has two components: one based on the transmission of the DNA sequence (arrow 1), and that whose avatar (arrow 4) is the epigenetic marks on the DNA (the 3D or even 4D dimension of the DNA molecule in gametes)[180].



A dazzling conclusion

In this chapter, I have taken the time to detail the mechanisms, without skirting certain technical aspects, as they are full of major lessons. On the one hand, these results are very surprising because of the great sharpness and sophistication of the mechanisms involved. On the other hand, they provide particularly convincing molecular arguments showing that parent-offspring resemblance on a large number of traits can be based on information of a non-genetic (non-sequencic) nature. *They thus provide astonishing evidence for the reality and pervasiveness of non-genetic inheritance.*

The Weismann barrier revisited

We are still far from understanding all the mechanisms responsible for these various forms of parent-offspring resemblance, and while in this chapter we have begun to address some of the issues we outlined at the end of the previous chapter, much remains to be done in this area. We will return to this on a conceptual level in **Chapter 16**. However, the results presented in this and the three previous chapters clearly show that there is a need for a thorough rethinking of the Weismann barrier concept[181]. It is clear that evolution has favoured the emergence of very sophisticated processes that allow to pass through this barrier, to the point that one may wonder whether this concept is still relevant today[182].

However, the examples of the inheritance of acquired metabolic diseases such as diabetes and the consequences of dietary deficiencies suggest that environmental effects are in fact only written into gametes *after meiosis*, during maturation, i.e. very late in gametogenesis, by injecting small non-coding RNAs of somatic origin into gametes (see **Box 2**). If this process were to be general, then the Weismann barrier concept would remain valid, and only relevant environmental information would then be injected back into the gametes. By this process, the environmental information somehow bypasses meiosis. However, it remains to be understood how the information carried by the small non-coding RNAs in the gametes manages to escape the waves of demethylation-remethylation that occur at the time of fertilisation. A simple solution would be that the gamete RNA information only begins to affect development after such epigenetic reprogramming. These are exciting avenues to explore.

A story of a blind painter

The incredible sophistication of the processes highlighted in the previous chapters cannot be the result of chance alone. Indeed, chance alone cannot generate complex processes. Let us imagine, for example, a blind painter who paints a landscape. Let us also imagine that he practices pointillism. Our blind painter would then place spots of colour randomly on the painting. It is unlikely that the painting would ever reproduce the complex structure of the concerned landscape, or even that it would ever be structured into something precise involving a complex and organised distribution of dots on the painting.

Suppose now that our blind painter had an associate who would erase all the dots put in by the painter when they were not the right colour in the right place. We can imagine that, after some time, the painting would begin to resemble the landscape concerned. In other words, the complexity of organisation and structure represented by a painting resembling reality cannot emerge from chance alone, but can hugely more quickly emerge from an association between a source of random variation (the coloured dots put in by the blind painter) and a filter that would retain only the correctly placed dots. The filter in this metaphor plays the role of natural selection, and the fact of being placed in the right place with the right colour plays the role of phenotypic fitness. The story of the blind painter is thus a metaphor of a mutation selection process.

*Processes that we predicted in **Erreur ! Source du renvoi introuvable.***

On the other hand, as we saw in **Erreur ! Source du renvoi introuvable.**, the existence of the inheritance of some environmental effects is expected in view of the selective advantage it could provide to organisms. So we should not be surprised; these findings only demonstrate the predictive power of evolutionary reasoning incorporating natural selection. And we will see in the following chapters (especially **Chapter 10**

Randomness and mutation

After discovering all these fascinating pathways of intergenerational information transfer, it is now necessary to develop an overlooked but basic property of epigenetic marks that is linked to a recurring issue in evolutionary biology, namely that of the randomness of mutations of all types. We have seen that one of the basic principles of the Modern Synthesis is that mutations are in no way directed by the environment towards improving the adaptation of organisms. Unfortunately, this principle is often simplified into saying that mutations occur at random, which does not mean the same thing. But what exactly is the case? This is what we will look at in this chapter.

Epigenetic marks are mutagenic...

The starting point that led me to think about the issue of mutation randomness was the fact that epigenetic marks, such as the presence of methyl radicals on cytosines, destabilises DNA and greatly increases the mutation rate of methyl-cytosines into thymine, another base of the DNA sequence. This, therefore, has the potential to generate point mutations whereby a cytosine is replaced by a thymine. Some articles have, for example, subheadings entitled "Methylation is mutagenic". For example, studies in humans suggest that cytosine methylation is responsible for 30-40% of point mutations in the human germline. Combining the results of several authors, cytosine methylation would increase the probability of cytosine mutating to thymine by a factor of about 20,000. This is such a considerable factor that it seems very unlikely that it is a negative collateral effect of a process selected in another context (in this case DNA methylation, which is involved in

the regulation of gene expression). What then could be the function of a process that destabilises the fidelity of sequencic transmission to such an extent?

This is what we addressed in a 2019 paper. We proposed a mechanism by which such mutagenic power of DNA methylation, and more generally of epigenetic marks, might have provided a real evolutionary advantage by accelerating the sequencic engraving of the initially plastic responses to environmental conditions that prove to be very persistent. We have given this mechanism the explicit but unmemorable name of *epigenetically-facilitated mutational assimilation*.

Genetic assimilation

The idea of *genetic assimilation* (see Glossary) was proposed by *Conrad Waddington* following a series of experiments in *Drosophila* showing that following an environmental stress triggering an initially plastic response, this response tends to become heritable (and therefore non-plastic) after a certain number of generations under the effect of this stress. It was therefore as if, after a few dozen generations, characters initially developed in a plastic manner in response to a given environment became 'genetically' engraved, hence the expression 'genetic assimilation'.

Genetic or epigenetic assimilation?

However, it should be noted that in this expression the term genetic was understood in its pre-DNA sense, as 'that which is transmitted', without prejudging the mechanism responsible for this transmission. In particular, while Waddington's experiments undoubtedly demonstrated that the initially plastic trait became inclusively heritable, they did not at all show that this necessarily implied a sequencic change. In effect, there was nothing in these experiments to suggest that what he observed at the phenotypic level resulted from a change in the DNA sequence. Given that Waddington had only worked over a few dozen generations —which was already a real challenge —he in fact most likely documented an "epigenetic assimilation" because the only thing his experiments really showed was that an initially plastic trait became inclusively inheritable within a few generations. This is equivalent to what *Mary Jane West-Eberhard* called "genetic accommodation" whereby a trait can be made heritable without necessarily involving encoding in the DNA sequence. Our paper proposed that, under certain conditions to which we will return later in this chapter, this process could go as far as sequencic engraving, *if the environmental stress persists over many, many generations*.

And the Modern Synthesis assimilated genetic assimilation

It has always puzzled me that the idea of genetic assimilation has finally been 'assimilated' by the Modern Synthesis, as this mechanism is strongly reminiscent of the much-rejected idea of inheritance of acquired traits. If you think about it, Waddington's mechanism proposes that within a few dozen generations under a given constant environmental stress the initially plastic response to stress can become heritable. In fact, what has allowed the idea of genetic assimilation to be assimilated is the relative slowness of this phenomenon. Moreover, the classical interpretation of this phenomenon is that there would pre-exist some neutral and hidden sequencic variation (usually called standing genetic variation) that would be somehow revealed by the environmental stress. Natural selection would then have the time to act over the few dozen generations of Waddington's experiments to retain only those variants that happen to be, I would like to say 'miraculously', favourable. So genetic assimilation would be just a special case of natural selection. This is how the Modern Synthesis has managed to see no major contradiction in genetic assimilation. This is also how I understood it until a few years ago.

Epigenetics as a hub towards sequencic engraving

A striking result on which we have built our reasoning is that all mechanisms of non-genetic heritability seem to involve some epigenetic change. It is as if epigenetics was the backbone or hub towards which most non-genetic inheritance processes would converge. Then, as epigenetic marks destabilize the DNA, over the course of many generations, this would generate sequencic variation *in the parts of the DNA concerned by the accommodation to the environmental change*. This would lead through natural selection acting on this newly produced variation, to sequencic engraving. In a way, epigenetics would be the conductor of the orchestra made up of all the genetic information. In effect, while it is very useful to have all the sequencic information (the recipe book), it is important to use it wisely. We shall see in **Chapter 16** that this epigenetic conductor is itself under the control of the brain.

With *Arnaud Pocheville*, then based at the University of Sydney in Australia, we modelled this idea and were able to show that such a mechanism could accelerate the transfer of epigenetic encoding to sequencic encoding by a factor of the order of magnitude of the mutagenicity of the epigenetic marks, i.e. about 20,000 times. *This is what we called the epigenetically-facilitated mutational assimilation.*

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But the story does not end there, as epigenetics interacts strongly with another major source of mutation, namely transposable elements.

... and interact with transposable elements

In parallel, we have been interested in another major phenomenon that can affect both the expression of certain genes and the appearance of mutations of all types. In fact, not only can the presence of epigenetic marks affect the stability of DNA, but epigenetic marks are themselves in strong interaction with the activity of transposable elements. Transposable elements are mobile DNA sequences discovered in maize by *Barbara McClintock* at the Cold Spring Harbor Laboratory on Long Island in the USA in the 1940s. This is one of the great genetic discoveries of the second half of the 20th century. There are a variety of transposable elements that differ, among other things, in the way they duplicate. Transposable elements exist in almost all living organisms. They seem to be able to invade the genome of an entire species through a process of colonisation from a local population, and can represent a large portion of the genome (about 15 to 22% in Drosophila, 40% of the genome in humans, and up to 90% in wheat). To give an idea of the prevalence of transposable elements, in humans, more than three million human sequences are derived from transposable elements, but only a few hundred of these have retained transposition capacity. The universality and mobility of transposable elements suggest that they play an important role in genome evolution and plasticity

The activity of transposable elements is under epigenetic control

The activity of transposable elements is strongly modulated by epigenetic processes (involving methylation, histone modifications or small RNAs) which are themselves affected by environmental factors. There are several hypotheses (not necessarily mutually exclusive) explaining the interaction between transposable elements and epigenetics. In particular, the targeting of epigenetic modifications to transposable elements could be a consequence of the *exaptation* (see Glossary) of transposable elements as platforms for chromatin modification, in which case the epigenetic regulation of transposable elements could be a consequence of genome defence and regulation. As a result, environmental stresses can trigger transposition activity, either directly or through their effects on epigenetic marks associated with transposable elements. It can be said that in most cases the mobility of transposable elements is inhibited by epigenetic marks that block their replication. However, this targeting of epigenetic marks on transposable elements also affects, as if by ricochet, the genes close to these transposable elements —with which they become partners in a kind of "transposable-element-gene duo"—, thus affecting their expression level. Beyond their important mutational effects, by duplicating themselves in the genome, transposable elements can thus affect the general functioning of the genome, among other things by regulating and controlling the activity of genes in the neighbourhood of their insertion point. Thus transposable elements affect gene activity in three different ways.

- First, by attracting strong epigenetic marking around their insertion point, they affect the epigenetic marks, and therefore the expression, of the genes with which they are in duo. It should be noted that the epigenetic marks around transposable elements can be modified by stresses bringing back their mobility, hence modifying the expression of the genes around the new insertion point.
- On the other hand, as the sequence of many transposable elements carries regulatory elements of response to the environment, their presence will directly modulate the expression of the genes with which they are in duo according to the environmental context. They therefore play a central role in the response to environmental changes.
- Finally, by their mobility within the genome, transposable elements can generate significant sequencic changes in the genome. Their mutagenic potential is thought to increase the average point mutation rate by several tens of thousands of times.

A great generator of inclusively heritable variation

Thus, the presence of transposable elements in one area of the genome can on the one hand durably modify the expression of the surrounding genes due to the strong intervention of persistent epigenetic marks inhibiting their mobility, and on the other hand generate genetic (sequencic) variation in the whole genome as a result of their mobility. Both types of variation can affect the phenotype either negatively for individuals (e.g. they are implicated in various diseases) or positively at the population level by generating variation that is inclusively heritable and therefore open to selection. In other words, while at the individual level these changes can often have negative consequences, at the population level transposable elements generate inclusively heritable variation on which natural selection can act, thus favouring the adaptation of populations to their environment.

Interactions between epigenetics and transposable elements thus constitute a real engine for the creation of phenotypic variation (targeted to specific portions of the genome) that can be inherited either sequentially or epigenetically *in response to environmental stresses*, and are thus an important factor in evolution. Such a generator of genetic and epigenetic variation can in particular explain changes in mutability within the genome

following environmental stresses. Several authors have emphasised the existence and importance of such generators of inclusively heritable variation involving the joint action of genetic and non-genetic processes in the ability of natural populations to adapt to ongoing global changes under the influence of human activities.

Epigenetically-facilitated mutational assimilation

We can now synthesize this. It appears that the effects of environmental stresses can affect the expression of specific genes involved in the response to stress and affect the activity of transposable elements, two major characteristics that each have the capacity to increase the sequencic mutation rate by tens of thousands of times, which is anything but negligible.

An information transfer pathway acting over many generations

The epigenetic changes affecting the expression of genes specifically involved in the response to an environmental stress in fact have two functions taking place on two very different time scale:

- First, these epigenetic marks, which we have seen target very precise portions of the DNA, enable the individual to adapt to the current environment by finely regulating the expression of the genes involved and leading to the phenotypic response to the environmental challenge. This response is rapidly established under the effect of environmental change. This process is known as phenotypic plasticity, the ability to modify the phenotype in response to the environment.
- Second, by being inherited, those epigenetic marks lastingly affect the mutability of the concerned genes that happen to be the genes involved in the accommodation to the specific environmental change. These epigenetic marks can also affect the activity of neighbouring transposable elements, which can further increase the mutability of the concerned regions and thus the potential generation of sequencic variation. In other words, epigenetic marking would differentially mark portions of the genome for mutation, i.e. for the generation of sequencic variation and thus for the multigenerational exploration of new genetic possibilities. Far from being a cost in terms of evolution, this may on the contrary constitute a major evolutionary benefit because the sequencic variation thus generated concerns the genes actually involved in the accommodation to the specific environmental stress, a variation then open to natural selection.

This is *epigenetically-facilitated mutational assimilation* that is more than just a special case of natural selection on initially neutral and hidden genetic variation suddenly revealed by environmental change. According to our view, genetic assimilation appears as *a genuine mechanism for manufacturing sequencic variation in the parts of the genome concerned by the accommodation to the specific environment*, variation which is then open to natural selection. This mechanism calls for several important comments.

Random mutations in environmentally targeted areas of the genome

First, with epigenetically-facilitated mutational assimilation, the fundamental axiom of the Modern Synthesis that *mutations are not influenced by the environment in an adaptive direction* remains 100% valid. However, it is the simplified phrase traditionally used to simplify this axiom "mutations are random" that appears incorrect. With epigenetically-facilitated mutational assimilation the mutations generated following a lasting environmental change are indeed not influenced in an adaptive direction by the environment (the axiom of the Modern Synthesis therefore remains valid), but the parts of the genome where the mutation rate increases are actually targeted by the environment. *This is because epigenetic changes and the activity of transposable elements are themselves targeted by the environment*. There are therefore two independent scales where randomness can be expressed, that of regional portions of the DNA, and that of the local change of sequence itself. Only the second scale is unaffected by the environment, whereas the regional scale is clearly targeted by the effects of the environment in the sense that it is precisely in the portions of the DNA concerned by the accommodation to the environmental challenge that the mutation rate changes.

A necessarily slow process…

Second, even if the magnitude of several tens of thousands of increase in mutation rate seems enormous, it does not mean that epigenetically-facilitated mutational assimilation (i.e. the sequencic engraving of the adaptation) takes place in a few generations. A rough calculation predicts that such a process must take hundreds, if not thousands, of generations to become effective. Although the calculation proposed in the last note is very crude, the important point is that we should not expect epigenetically-facilitated mutational assimilation to take place very quickly, and certainly not in only a few tens of generations. And in fact, evolutionary logic even leads us to believe that this slowness is integral to the process (see below).

… which could be involved in domestication

We were certainly not the first to think about this type of genetic assimilation where the environment can be involved in generating genetic variation in the sections of the genome involved in the response to the

environment. For example, one of the earliest papers on the subject dates back to 1983 in which *Hugh Iltis*, then Professor of Botany at the University of Wisconsin, formalised a scenario for the domestication of maize from teosinte, an annual plant from Central America. This remarkable scenario integrated several previous hypotheses and involved the major and massive effect of what he called a catastrophic epigenetic sexual transmutation that occurred some seven millennia ago.

Similarly, the whole literature on transposable elements claims that the environment can generate inclusively heritable variation. Regarding the idea that the environment can generate variation in certain regions of the genome, *Eva Jablonka* and her collaborators had modelled this idea without proposing a molecular mechanism. Similarly, *Michael Skinner* also foresaw and proposed the existence of such phenomena. Furthermore, researchers working on the domestication syndrome of vertebrates proposed that the stress induced at the beginning of domestication must have caused alterations in the methylation patterns of developmental genes expressed in the neural crest (the part of the embryo that will become the central nervous system), epigenetic changes that could have been fixed in the form of genetic variants to explain recurrent behavioural resemblances in the many domesticated fish, mammals and birds.

The different systems of inheritance interact with each other

This chapter thus introduced a particularly important point, namely that the different systems of inheritance (which we will summarise in **Chapter 15**) do not operate independently of each other. On the contrary, they interact and influence each other. For example, the central idea of epigenetically-facilitated mutational assimilation is that the molecular memory represented by epigenetics states interacts over the long term with sequencic memory, in a way that can potentially considerably accelerate the genetic encoding of initially plastic responses to environmental characteristics that persisted for hundreds or thousands of generations.

Chapter 11 andChapter 15), that there are also many other non-sequencic processes of inheritance that are effective in producing parent-offspring resemblance, and that are of a completely different nature from any of the non-sequencic processes of inheritance we have seen so far. So more discoveries await us.

A surprising conclusion, to say the least

In the same vein, there is the question of the type of acquired environmental effects that can be transmitted to offspring in this way, a question made more glaring by the impressive specificity of this type of transmission (being afraid of one smell but not another). Are these effects only physiological constraints (as there must be many in the case of stress), or are the inherited acquired traits naturally selected[183]? These are all fascinating questions to which we will return in the third part of this book.

The generality of the phenomena described above seems to contradict certain foundations of the Modern Synthesis[184] that, for instance, does not admit the existence of the transmission of acquired characters, a form of heredity that it considers as soft. However, this contradiction may only be apparent. At this point it should be realised that principles laid down almost a hundred years ago to simplify our approaches need of course be revisited in the light of recent discoveries and we should not transform them into immutable dogmas. Simplification is at the heart of science, but only for a certain amount of time, and we must not forget that our models are mere simplifications of reality. The facts must always have the last word. The time for the sequencic simplification is clearly over.

Chapter 10
Randomness and mutation

After discovering all these fascinating pathways of intergenerational information transfer, it is now necessary to develop an overlooked but basic property of epigenetic marks that is linked to a recurring issue in evolutionary biology, namely that of the randomness of mutations of all types. We have seen that one of the basic principles of the Modern Synthesis is that mutations are in no way directed by the environment towards improving the adaptation of organisms. Unfortunately, this principle is often simplified into saying that mutations occur at random, which does not mean the same thing. But what exactly is the case? This is what we will look at in this chapter.

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Epigenetics as a hub towards sequencic engraving

A striking result on which we have built our reasoning is that all mechanisms of non-genetic heritability seem to involve some epigenetic change[197]. It is as if epigenetics was the backbone or hub towards which most non-genetic inheritance processes would converge. Then, as epigenetic marks destabilize the DNA, over the course of many generations, this would generate sequencic variation *in the parts of the DNA concerned by the accommodation to the environmental change*. This would lead through natural selection acting on this newly produced variation, to sequencic engraving. In a way, epigenetics would be the conductor of the orchestra made up of all the genetic information. In effect, while it is very useful to have all the sequencic information (the recipe book), it is important to use it wisely. We shall see in **Chapter 16** that this epigenetic conductor is itself under the control of the brain.

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But the story does not end there, as epigenetics interacts strongly with another major source of mutation, namely transposable elements.

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In parallel, we have been interested in another major phenomenon that can affect both the expression of certain genes and the appearance of mutations of all types. In fact, not only can the presence of epigenetic marks affect the stability of DNA, but epigenetic marks are themselves in strong interaction with the activity of transposable elements[198]. Transposable elements are mobile DNA sequences discovered in maize by *Barbara McClintock* at the Cold Spring Harbor Laboratory on Long Island in the USA in the 1940s[199]. This is one of the great genetic discoveries of the second half of the 20th century. There are a variety of transposable elements that differ, among other things, in the way they duplicate[200]. Transposable elements exist in almost all living organisms. They seem to be able to invade the genome of an entire species through a process of colonisation from a local population[201], and can represent a large portion of the genome (about 15 to 22% in Drosophila[202], 40% of the genome in humans, and up to 90% in wheat). To give an idea of the prevalence of transposable elements, in humans, more than three million human sequences are derived from transposable elements, but only a few hundred of these have retained transposition capacity[203]. The universality and mobility of transposable elements suggest that they play an important role in genome evolution and plasticity

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A great generator of inclusively heritable variation

Thus, the presence of transposable elements in one area of the genome can on the one hand durably modify the expression of the surrounding genes due to the strong intervention of persistent epigenetic marks inhibiting their mobility, and on the other hand generate genetic (sequencic) variation in the whole genome as a result of their mobility. Both types of variation can affect the phenotype either negatively for individuals (e.g. they are implicated in various diseases) or positively at the population level by generating variation that is inclusively heritable and therefore open to selection. In other words, while at the individual level these changes can often have negative consequences, at the population level transposable elements generate inclusively heritable variation on which natural selection can act, thus favouring the adaptation of populations to their environment[208].

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Similarly, the whole literature on transposable elements claims that the environment can generate inclusively heritable variation. Regarding the idea that the environment can generate variation in certain regions of the genome, *Eva Jablonka* and her collaborators had modelled this idea without proposing a molecular mechanism[215]. Similarly, *Michael Skinner* also foresaw and proposed the existence of such phenomena[216]. Furthermore, researchers working on the domestication syndrome of vertebrates proposed that the stress induced at the beginning of domestication must have caused alterations in the methylation patterns of developmental genes expressed in the neural crest (the part of the embryo that will become the central nervous system), epigenetic changes that could have been fixed in the form of genetic variants to explain recurrent behavioural resemblances in the many domesticated fish, mammals and birds[217].

The different systems of inheritance interact with each other

This chapter thus introduced a particularly important point, namely that the different systems of inheritance (which we will summarise in **Chapter 15**) do not operate independently of each other. On the contrary, they interact and influence each other. For example, the central idea of epigenetically-facilitated mutational assimilation is that the molecular memory represented by epigenetics states interacts over the long term with sequencic memory, in a way that can potentially considerably accelerate the genetic encoding of initially plastic responses to environmental characteristics that persisted for hundreds or thousands of generations.

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Chapter 11
Cultural inheritance

Do you know people who do not speak the language of their parents? I don't know any. And yet, in my job, which is very conducive to meetings between people from different countries, I have experienced some very unusual situations. For example, I have an Italian colleague, married to a Dutch woman and living in France. Their offspring speak French, of course, but also Dutch and Italian, which their parents have used since childhood. Similarly, the offspring of a friend couple who are English and Spanish are fluent in both languages. They also speak French because they lived for some time in Luxembourg. Amusingly, there is variation among their three children. The eldest is close to his father and prefers English, the second prefers French as she had her secondary education in Luxembourg, and the third prefers Spanish, the language of the country of her childhood. As a final example, both parents of a friend of mine during my studies in Paris were French, but the father being a diplomat he grew up mainly in Germany, where he attended an international high school, where classes were taught either in French, English or German. This young man was of course fluent in all three languages.

These examples highlight three major facts: offspring always speak the language of the parents they grew up with; one can learn several languages easily early in life; people also speak the language of the environment in which they grew up. This is another form of heredity that leads to very high heritability (which we have seen estimates the level of parent-offspring resemblance), very close to 1, a value that is rarely reached for traits considered as genetically encoded.

Yet we all know that this parent-offspring resemblance is based on completely different mechanisms than those we have discussed so far. The previous chapters showed how necessary it is to broaden our vision of heredity. Today, we can no longer ignore the immense capacity of living organisms to engrave and transmit information whose subtlety and reactivity to the environment go far beyond the properties of sequencic information alone. In fact, all the examples and reflections developed in Erreur ! Source du renvoi introuvable. to **Chapter 9** show that DNA carries many other types of information through epigenetic marks affecting its 3D (or even 4D) configuration, which durably modifies the expression of genes and change the phenotype of individuals and their descendants over several, or even many generations. Of course, the information embedded in the DNA sequence is important, but it must be used judiciously and this depends largely on the environmental conditions prevailing at a given time.

All these results, which you will have noticed have emerged mainly since the beginning of the third millennium, are fascinating indeed, but in this chapter I want to develop another equally fascinating form of inheritance, of a completely different nature from those we have been talking about so far. This is cultural transmission, a transmission that can lead to behavioural as well as morphological or physiological variation, and which, being made heritable by social learning, becomes open to natural selection.

Even today, cultural inheritance is still too often ignored partly because it is based on purely cognitive mechanisms for which we do not necessarily know the molecular or neurological bases. It is thus often considered secondary. The study of cultural inheritance has therefore unfolded in parallel and independently of the study of epigenetic inheritance that we essentially discussed up to now. These two fields have simply ignored (which, unfortunately, in science often means despised) each other. Yet the history of the study of cultural transmission begins long before that of epigenetics, since it is clearly mentioned in the writings of Darwin (1859) and later by Baldwin (1896) among many others.

A brief history of the study of the cultural phenomenon

For *Charles Darwin* social transmission leading to parent-offspring resemblance is integral to the concept of heredity[218]. That book is full of examples that clearly fall within the scope of what is now called cultural inheritance.

As for me, when, in 2013, I submitted and obtained from the French National Research Agency[219] a research project that I called *Social Heredity* (or *Soc-H²*) to fund my research until 2018, I was particularly proud of the title I had invented for this project. I was amazed to discover some time later that the same phrase 'Social heredity' was the title of a section of an article by *J. Mark Baldwin* published in 1896[220]. Beyond the fact that this shows how often we are just reinventing ideas of our predecessors, it shows how much social (or cultural) heredity was integral to heredity in the late 19th century. As we saw, the discovery of DNA and the

genetic code led the entire scientific community, fascinated by this incredible discovery, to reduce inheritance to the mere transmission of the DNA sequence[221].

However, as always in science, even if not always taken seriously, the facts are stubborn. We will look at a few examples that are classically used to illustrate the reality of cultural transmission outside of humanity alone. The two most often cited examples are the chickadees in the UK and the Japanese monkey.

Premises in animals...

Chickadees stealing cream from milk bottles

In the old days in Britain, the milkman would leave a bottle of milk on his customers' doorsteps every morning. In 1921, the inhabitants of a village in England reported that birds described as tits had taken to piercing the foil lids of the bottles in order to feed on the cream floating on the milk[222]. This behaviour was a real innovation that apparently occurred in several places and then spread throughout the British Isles at a speed incompatible with that of a hypothetical mutation that then spread by the movement of individuals. This example has been the subject of much debate, particularly about the nature of the learning responsible for this rapid spread[223], as it was one of the first examples described of an innovation that then spread by social learning[224]. There was also much debate as to whether or not this could be considered a cultural process. This raises the question of how to define the cultural process, which is the focus of this chapter.

Macaques washing their food

In the early 1950s, Japanese researchers began studying a troop of Japanese macaques living on a small island called Koshima[225]. In 1952 they placed sweet potatoes at various locations to attract the monkeys to open areas outside the forest. In 1953 they observed that a young female called *Imo* (which means potato in Japanese) washed the potatoes in the river before eating them. This removed all the sand and soil that stuck to the potatoes, making them easier to eat. This innovation then spread to almost all troop members. The monkeys then started carrying potatoes with them and began washing them in sea water, which had the advantage of salting them at the same time.

Later, the researchers cunningly dropped wheat on the beach. While they thought the monkeys would take a long time to collect the grains one by one, they observed that the same female *Imo*, now 4 years old, quickly solved the problem by throwing a mixture of sand and wheat into puddles of sea water. Being twice as heavy as water, the sand sank to the bottom while the wheat floated to the surface and could therefore be skimmed off easily. This innovation was again adopted by the other members of the group, starting with the youngest. This led the monkeys to visit the beach, and the young accompanying their mothers soon developed the habit of bathing in the sea, a new habit which was later adopted by adults. Some individuals began to catch and feed on fish.

In short, the experiment conducted by the researchers completely changed the living habitat and all the habits, especially the eating habits, of all the members of this troop of monkeys in a few years. New traditions emerged and accumulated over time as they were adopted by all group members.

And several vertebrates that display cultural behaviour

For a long time these two examples were the only two examples of animal culture alongside with the existence of song dialects in bird, which today still constitute one of the best documented example of culture and cultural evolution in non-human animals[226]. However, in recent decades many other examples have been described and well documented. One example is the study of Chimpanzees across tropical Africa showing that the behaviour of individuals in various situations differs consistently between separate populations[227]. Serious arguments have been published in Orangutans, vervet monkeys, several cetaceans, meerkats, and various bird species. Arguments have even been made recently in insects[228].

...in parallel with theoretical approaches in humans

Well prior to this slow emergence of empirical arguments[229] in favour of the existence of animal culture, human sciences had made considerable progress on the question of the importance of cultural transmission for human evolution. For obvious reasons these approaches were essentially conceptual or theoretical. These many approaches were designed to study the particular case of the role of culture in the evolution of our species. In doing so, they were ahead of all other sciences in addressing the role of cultural processes in evolution[230]. As a result, human sciences were quickly led to assert that the pure sequencic view of heredity is not sufficient in explaining the evolution of the human species. In doing so, they pioneered the domain.

These approaches showed many important things. For example, one theoretical study showed that the social transmission of sexual preferences alone can drive an initially rare male trait to fixation, even if it has a real negative effect on male viability[231]. These studies have also shown the importance of *conformity* (see

Glossary) in social learning for the emergence of genuine local cultural traditions[232]. *Conformity refers to the tendency of individuals in a group to adopt disproportionately the most common behaviour in the group*[233]. In other words, individuals tend to adopt the behaviour of the majority of individuals in their group.

Human sciences have done such a good job of advancing the study of culture that at the turn of the third millennium we had far more theoretical arguments for the existence of culture than empirical arguments based on rigorous observations, let alone experimental studies. Since then, concrete arguments for the existence of cultural processes in a wide variety of vertebrates but also more recently in invertebrates have been published.

Defining animal culture

One might legitimately ask whether these examples are all that important. The impression that emerges from the relative rarity of cases of cultural transmission in animals could lead one to think that they can be disregarded as a series of small exceptions. However, it should be noted that in science, as in everyday life, we only find what we are looking for, and for that goal to be achieved, we need to know exactly what we are looking for.

The fact that it was the human sciences that initiated the study of cultural processes had the unfortunate consequence of reinforcing the common belief that cultural processes exist only in humans. And in fact, most definitions of the cultural process were designed to capture the highly original particularities of human culture instead of focusing on the essence of the cultural process itself. As a result, for a long time, animal behaviour sciences were not concerned with the question animal culture. In fact, there were two major errors in this attitude.

- First of all, as soon as a process leads to parent-offspring resemblance, it becomes a *de facto* actor of evolution. And the fact that this process is rare certainly does not mean that it can be neglected. We have already discussed the weight of rarity in Erreur ! Source du renvoi introuvable.
- On the other hand, the question of the definition of culture was central to the debate. To study animal culture we need a definition of culture that focuses on the essence of culture rather than on the particularities of human culture. After all, if the cultural process is defined in a human-centred way, there is no reason to investigate it in animals.

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Thus, the question of how to define animal culture in general was key for the emergence of the study of animal culture. What do we mean by animal culture? What is in fact the basic process of culture?

Needless to say, this issue has been and still is the subject of endless debate. In April 2019, I was invited to a week-long seminar in Leiden, the Netherlands, on the foundations of cultural evolution. The seminar mixed researchers from human and evolutionary sciences. The organisers had asked us in advance of the meeting to give our definition of cultural evolution. It was striking to see how the collected definitions emphasised things of a totally different nature. Some of them talked mainly about institutions, arts and other human specialities. Although this might make it seem that the discussion would be particularly difficult between these two fields, my experience is that the idea of putting people from different backgrounds in the same room can really help to move things forward and bring people from different disciplines to work together.

A USB-BMW story

To illustrate my point, here is a metaphor. In 2009 on my way back from Anchorage, on the plane between Amsterdam and Toulouse, I was sitting next to two young dynamic executives. They discovered that they had the same BMW. One asked the other "Do you have the USB version?" Tired from 24 hours of travel, I started to imagine a huge USB stick in the shape of a BMW. Then I realised that there had to be a USB port in the vehicle to plug in your phone or other musical devices. At the time I had never heard of USB ports in cars because they existed in luxury vehicles only.

Now imagine an alien who lands on Earth to discover a new and fascinating concept, that of a car. By chance he came across a USB-BMW first, so he sets about defining this concept by talking about the engine, the wheels, the steering wheel, etc. and the USB port. As he is a perfectionist, according to his definition all these elements are necessary to be able to call something a car. Thus, according to our alien, if there is no USB port, we cannot talk about a car.

The problem, of course, is that we didn't wait for this alien to invent this concept, so we're going to argue with him to make him understand that what defines a car is not the steering wheel, nor the engine and even less the USB port, but something much more basic: the wheel. The car concept was created with the invention of the wheel. The most primitive form was a board on wheels, which you pulled yourself (a handcar), or had pulled by cows, horses, or increasingly sophisticated engines, but the basic concept that defines a car is the wheel. We should not, therefore, under the pretext that we have invented, eventually in the course of a very long evolutionary history, objects as fascinating as our present-day cars, forget that historically all that we call a car has in common this increased mobility thanks to the wheel.

The parallel with culture is obvious: we need to search for and study the most basic concept that defines the cultural process, which does not prevent us from also studying its more evolved and fascinating forms, such as those we observe in our species. *There is no contradiction.* So, in order to study the cultural phenomenon, its appearance in the course of evolution as well as its complexification and impact in the course of evolution, we need to use a definition focusing on the most basic process that differentiates culture from all other processes. In a way, we need to find the wheel of culture.

A definition focused on the essence of the cultural process

I don't think I'm shocking anyone by saying that culture is about traits that we have learned from other people. The basic process of culture is therefore social learning, that is, learning from others[234]. *Thus the wheel of culture is social learning.*

Social learning

The literature is full of examples of social learning in vertebrates and insects or arthropods. One of my colleagues in Toulouse, *Audrey Dussutour*, has even identified a simple form of learning in a single-celled organism that is strikingly similar to social learning[235]. There are entire books on animal social learning. It is therefore an important research topic worldwide.

Thus, the essential process that can lead to culture exists in a very large range of species belonging to many taxa. But can we deduce from this that the cultural phenomenon is equally ubiquitous in animals?

Is social learning enough?

One can quickly grasps that the mere fact that a behavioural pattern is socially learned is not enough to bring about a real cultural process, because intuitively, one perceives that to be cultural a trait must be common to a whole group of individuals and must persist intergenerationally. In other words, an important dimension of the cultural process is that it must give rise to *traditions*, i.e. collective habits of behaving in the same way in a given situation. Culture is thus a group property. This common attitude may differ from one group to another, and then we can speak of *local traditions*.

The importance of the existence of local traditions

This concept of tradition is at the heart of most of the empirical studies of the cultural processes that I have briefly described above. It is also the case of the magnificent trans-African study of many Chimpanzee populations[236] that shows that in the same circumstances (e.g. to eat a seed with a solid shell), in a given region, chimps adopt the same behavioural technique (e.g. breaking the shell with a stone), whereas in another population in the same situation the monkeys break the shells with a wooden club.

The vast majority of studies revealing the existence of animal culture to date describe the existence of persistent behavioural variation among populations of the same species. These studies therefore adopt a definition of culture that focuses solely on the existence of patterns of behavioural variation between populations. This seems to make sense. But is it sufficient?

A certain persistence

An important aspect of the existence of animal traditions is the persistence of common behaviour. A tradition can only be said to exist if the collective behaviour persist over time, i.e. if individuals in a population persist in behaving in the same way over time when confronted with a given type of situation. All studies reporting the existence of cultural traditions have explicitly or implicitly incorporated this important feature.

But are patterns of tradition enough?

Documenting the existence of persistent patterns of behavioural variation among populations is an excellent first step in the study of animal culture, but it is not sufficient. In particular, observed behavioural differences may be due to either genetic or environmental variation among populations. It could be that different populations have different alleles that lead them to behave differently. Similarly, these populations could live in environments that are sufficiently different that the most appropriate behaviour is reinvented by each individual in the absence of social learning. For example, in one habitat the absence of stones may lead to the use of clubs to break fruit shells. In which case each individual could reinvent the same technique without having learned it from others. Hence, we would not be able to talk about culture.

It therefore appears that, as important as it is, an approach documenting patterns of behavioural variation among populations can only be a step towards demonstrating the existence of a genuine cultural phenomenon. We also need to demonstrate that the observed variation result from a form of social learning.

An approach focusing on the properties of social learning

Mate copying

In the early 1990s, emerged the study of social learning in the context of sexual mate choice[237], as it appeared that, after having seen a female (called demonstrator) prefer a given male to another, a female (called observer) having witnessed this choice now shows a preference bias for the male preferred by the demonstrator female. As it is as if the observer females copy the choice of the demonstrator females, this type of learning has been termed 'mate copying'[238]. Since the 1990s, experimental evidence for mate copying has been provided in a large number of fishes, birds and mammals (including humans) as well as in at least one insect species[239]. It thus appears to be a very general phenomenon.

At the beginning, several authors having documented mate copying claimed to have identified a case of cultural transmission. However, as we have just seen, although social learning is a necessary condition for cultural transmission to take place, it is not a sufficient one. This led to the identification of other necessary conditions for cultural transmission. In 2010, with my colleague *Richard H. Wagner*, we identified three criteria in addition to social learning that are necessary for the emergence of a culture[240]. In our paper we introduced a definition of animal culture that did not focus solely on the existence of tradition. Rather, our definition focused on the properties of social learning that can give rise to cultural traditions. I then took up and developed that definition in 2011[241]. However, at that time we had only identified four criteria and had missed a fifth criterion that turned out to be very important. We corrected this error in 2018[242]. Here are these 5 criteria.

- *Criterion 1: The trait must be learned from others*. For any trait to be cultural, it must be socially learned, i.e. acquired by copying, imitating or learning from others. This is the essence (or the wheel) of culture.
- *Criterion 2: The trait should be transmitted between age classes*. For this trait to persist over time, it must be regularly transmitted from older to younger individuals. This can be from parents to offspring, but also among neighbours. Without this condition, for example if the transmission is only among individuals of the same age, then the trait would only persist until the disappearance of that cohort and it would have to be reinvented with each generation. There would therefore be no long-term transmission.
- *Criterion 3: The trait must be memorised*. In order for the trait to be culturally acquired, the socially learned habit must be memorised for a sufficient period of time so that it can be copied by other individuals. In other words, only those behaviours that are held on to are transmitted socially.
- *Criterion 4: Social learning must be about a trait and not about a specific individual or situation*. This fourth criterion is more subtle. If, in a mate copying experiment involving male A and male B, it is found that, following the observation that a demonstrator female prefers male A, the observer female shows a bias for A over B, mate copying is said to be individual-based. In this case, this learning is interesting but cannot be the basis for cultural transmission because its effects can only persist for the lifetime of males A and B. In contrast, if mate copying leads observer females to prefer any male with the traits of A then this is called trait-based mate copying. It is only in this case that the socially acquired preference for males of the phenotype of male A can be transmitted over many generations[243]. In my 2010 and 2011 articles defining animal culture, the list of criteria ended there. We have since realised that we had missed a fifth criterion of major importance.
- *Criterion 5: Social learning should be conformist*. This last criterion exactly corresponds to the 'normalisation' developed by Dawkins in his letter cited in the Forewords of this book. Conformity is a mechanism of normalisation. In effect, in the wild, young females reaching maturity do not see a single female choosing between two types of males, as in our experiments, but see many females older than themselves choosing males with either trait A or trait B. What can they learn in such a case? It is hard to say. However, one could predict it by assuming that *females learn to prefer males of the phenotype preferred by the majority of females in the population*, that is, if females are conformist in their social learning. If social learning is conformist then, even if some individuals in the population behave in an original (i.e. less common) way, from generation to generation most individuals will behave in a given way. There will then be a true cultural tradition, i.e. a collective preference, with a majority persistently adopting a given behaviour to the detriment of other possible behaviours in that situation. And we have seen above that the existence of such traditions is the main marker of the cultural phenomenon.

A life-size application

We thus finally had a convenient definition of animal culture that focused on the essence of the transmission process, i.e. on the properties of social learning that can give rise to local traditions. The next step was to test this definition on a given biological system. We did this using a species that was *a priori* highly unlikely to be used for the study of animal culture, namely the fruit fly *Drosophila melanogaster*.

I started working on this species for a reason that has nothing to do with science. As my countless applications to fund my research were unsuccessful[244], I decided in the late 1990s to start working on the fruit fly as it was particularly cheap. By investing about 1000€ per year from my own pocket, I could carry out interesting experiments on the behaviour of these flies. As at that date, most of my career had been based on observational approaches without any experimental manipulations, I had to learn how to design experiments relevant to the study species. This took me about 10 years. In the early 2000s, while having lunch with one of my PhD students, *Susana Varela*, I came up with a crazy idea. I had been interested in mate copying for a few years and I said that we could test this in the fruit fly. Susana, who is always enthusiastic, immediately wanted to try this idea. Despite my assertions that this was not a serious idea, she designed and started such an experiment[245]. I doubted that it was possible that a fly whose brain is between 1 and 10 million times smaller than ours could have the cognitive abilities to collect and use such subtle *social information* (see Glossary). As we will see in this section, Susana and all the students I have had since have shown that I had unduly underestimated fruit flies and, beyond that, the power of natural selection.

Fruit flies can mate copy

The facts proved Susana right. Fruit flies proved to be able to perform mate copying. I once presented her results in Toulouse and someone in the audience told me that *Frédéric Mery*, who works at *Gif sur Yvette* and whom I know very well, had also found this type of result in that species. As I had not been able to find any trace of such results in the literature, I called him and we decided to publish our two experiments together, which was done in *Current Biology* in 2009[246].

Doubt is part of research

This result seemed too good to be true. But since then we have replicated this result many times, on different strains of *Drosophila*, by different students, over different years, with different experimental set-ups. The conclusion today is that there is no longer any room for doubt, fruit flies can perform mate copying. However, I was only at the beginning of my surprises concerning their cognitive capacities.

The 2009 article reported the results of our experiment and that of *Frédéric Mery*. I must confess that Frédéric's protocol was much more effective and convincing than ours. As Frédéric did not want to continue on the topic of the mate choice (he had been working on feeding and laying site choices for a long time), we agreed that we would use his protocol to continue studying this surprising phenomenon.

At that time, my objective for a few years had been to test the new definition of animal culture (published in 2010) on a single system that was relatively easy to work with. The idea was therefore to test on that species the four and then five criteria we saw above. It took us several years to define and master the numerous protocols needed to achieve this objective. During this long period I regularly doubted that Drosophila had the cognitive abilities to fulfil all these criteria. Facts proved me wrong.

Fruit flies meet all five criteria

Based on this first surprising result, and despite my doubts, *Guillaume Isabel* and I decided to investigate whether the behavioural trait of female sexual preference was likely to be culturally transmitted across generations in *Drosophila*. This project was explored from 2011 onwards by a PhD student, *Anne-Cécile Dagaeff*, and then implemented by our post-doctoral student, *Sabine Nöbel*, who carried out most of the experiments, the rest having been obtained by several students all under her kind and efficient supervision.

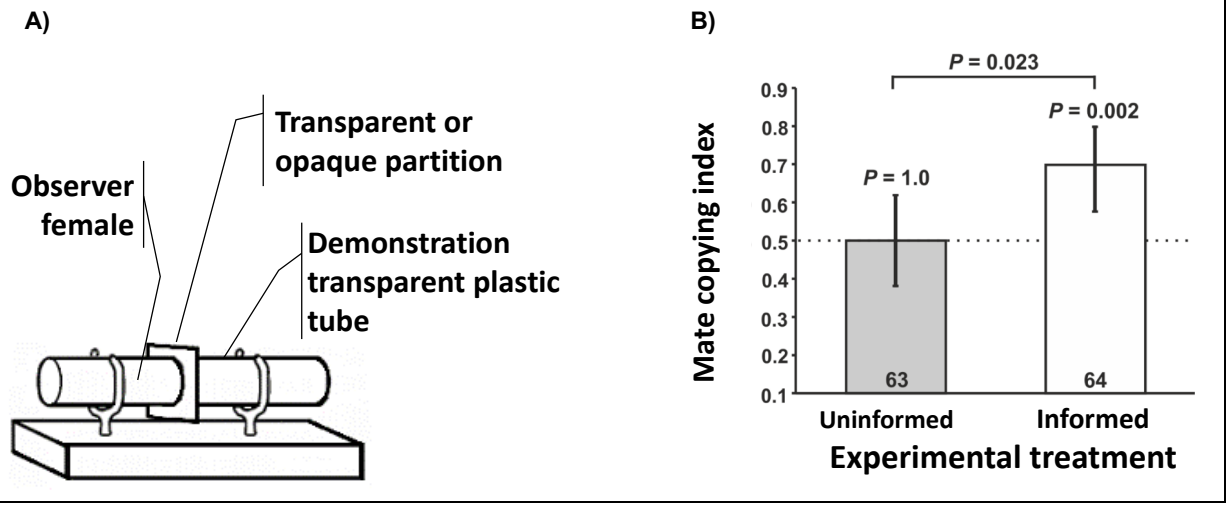
The basic experiment

For the main experiment we created contrasting male phenotypes by dusting them with green or pink powders (thereafter called green and pink males). The experiment takes place in a device consisting of two transparent plastic tubes separated by a partition which can be either transparent or opaque (**Figure 9, A**). It has two phases, a "demonstration" followed by a "mate choice test". In the protocol published in 2009 the demonstration lasted 3 hours and involved 6 successive phases. It was simplified by *Anne-Cécile Dagaeff* during her PhD, who in comparing the former protocol with a simplified one involving a single demonstration of a virgin female choosing between a green and a pink male, found no significant differences between these two protocols. The demonstrator female copulates readily with one of the males, thus providing positive information for the male she is copulating with and negative for the other male. This new protocol divided the duration of the demonstrations by a factor 6 and the number of flies needed by a factor 8, thus making our experiments much lighter. We called this 'speed learning', referring to the expression 'speed dating'[247].

Anne-Cécile introduced me to *Guillaume Isabel* at the beginning of her PhD. Guillaume is a specialist in the neurobiological mechanisms of memory in *Drosophila*. So we were bound to work together. From then on, everything accelerated, especially with the recruitment of *Sabine Nöbel* as a post-doctoral student in charge

of the project. Later *Arnaud Pocheville* wrote the computer program to study the potential of mate copying to foster the emergence of local traditions.

Figure 9: The basic mate copying experiment in Drosophila. A) Experimental set-up developed by *Simon Blanchet* during his post-doc with me. A transparent partition allows the observer female on one side to see the demonstration taking place in the other compartment. Under these conditions the observer female is informed. With an opaque partition the observer female is uninformed because she cannot see the demonstration taking place in the other compartment. **B)** Results of the test for criterion 1. The mate copying index is the percentage of observer females copulating with the male of the colour chosen by the demonstrator female during the demonstration. The horizontal dotted line at 0.5 visualises the expectation if the observing female copulated randomly. Above this line, it indicates that the observer females showed a tendency to copulate with the male of the colour chosen during the demonstration. The numbers in the bars give the sample sizes tested in the concerned experimental condition. The P-values above the bars indicate the probability of being wrong in stating that the result differs from a random choice. The probability above the horizontal bar is the probability of being wrong in stating that the two bars differ from each other[248].



Criterion 1: Social learning of sexual preferences

To test this criterion, we repeated Anne-Cecile's experiment with the same conclusion, after seeing the demonstration, observing females showed a bias for males of the colour chosen in the demonstration (**Figure 9, B**). This confirmed that criterion 1 was indeed met[249].

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Criterion 2: The trait is transmitted across age classes

To test this criterion, we slightly modified the basic protocol by changing the age of the demonstrator flies. Since all our experiments were conducted on 3-day-old virgin flies, and knowing that the fly larval development in the laboratory takes 11 days, we used 14-day-old demonstrator flies, which corresponds to the minimum age of the parents of the observer females. We found a result very similar to the previous one, thus showing that social learning also exists from older flies to younger flies. Criterion 2 was therefore met.

Criterion 3: Socially learned sexual preference should be remembered in the long term

To test this criterion we relied on all the knowledge of *Guillaume Isabel* on *Drosophila* long-term memory. The testing of this criterion required rather heavy experiments that were part of *Magdalena Monier*'s PhD. She showed unambiguously that *Drosophila* can build a long-term memory involving *de novo* protein synthesis. Long-term memory is tested at 24 hours (which represents a significant portion of the life of an adult *Drosophila* that is supposed to live for about a week in the wild). Thus criterion 3 is also met.

Criterion 4: Social learning is trait-based, not individual-based

We had several arguments suggesting that females in the previous experiments were not learning to prefer a given male over another male, but males of a given colour. For example, in all of the above experiments[250] we used different males for demonstrations and choice tests. This suggested that the females did learn to prefer a colour and not a particular individual. However, one could argue that the females may have confounded males of the same colour used in the demonstration and the choice test. We therefore conducted a series of experiments to test this criterion[251]. The idea was to use in the choice tests green and pink males with a mutation that gave them a very different and therefore unmistakable look compared with the wild-type males used in the demonstrations.

The conclusion was unambiguous in that the females had indeed learned to prefer males of one colour, showing that their social learning was actually based on traits and not on individuals and that therefore criterion 4 is also met.

Criterion 5: The social learning must be conformist

Testing this fifth criterion raised a technical challenge. We had to show that observer females learned the most frequently expressed preference amongst a group of demonstrator females. We therefore needed to show several demonstrator females each choosing between a green and a pink male to observer females. We also needed to manipulate the majority, which required a series of technical adjustments because we wanted to decide ourselves which colour the demonstrator females would choose, so as to avoid any other uncontrolled confounding factors. All these adjustments involved three students, *Adeline Loyau* who was the first to sense the importance of conformity, *Anne-Cécile Dagaeff* who developed a new device *and Sabine Nöbel* who masterfully set it to music to obtain, among other things, the following results.

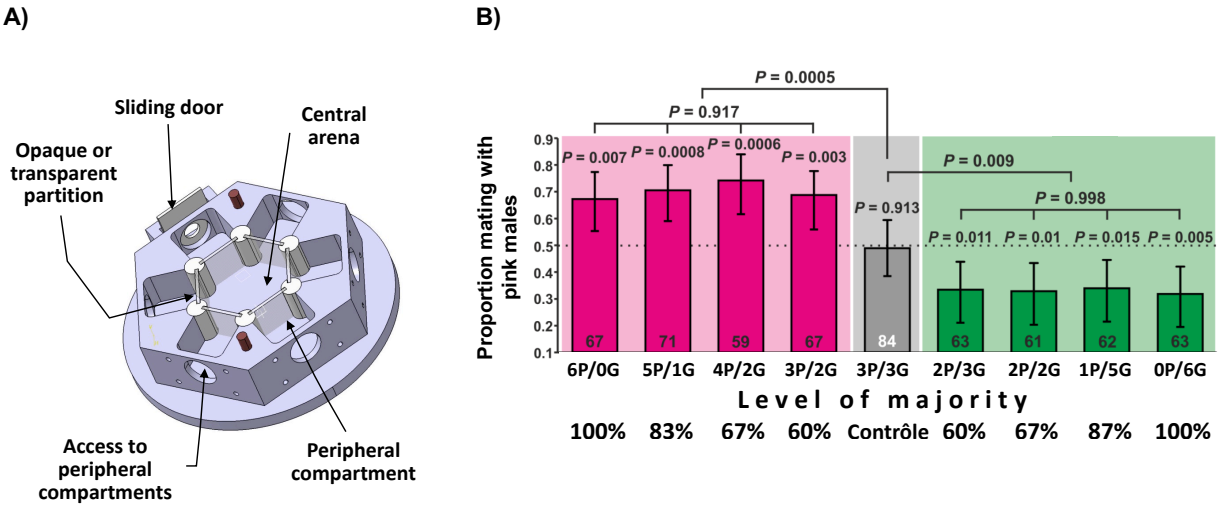
For this purpose we developed a new experimental device comprising a central arena in which the observer females are placed, surrounded by six independent compartments, in each of which unfolds a demonstration consisting of one demonstrator female copulating with a male of a colour chosen by us, plus a male of the other colour apparently rejected by the demonstrator female (**Figure 10,A**). We called this new device the hexagon (although my colleague *Philipp Heeb* dubbed it the 'inverted peep show', for reasons I will let you guess[252]).

With this hexagon, we could vary the majority from 100% down to only 60% with two intermediate values. This made four levels of majority for each colour. We also had the possibility to have a control where 3 of the demonstrator females copulated with males of one colour and 3 with the other. The results of this experiment surprised us so much that we repeated it (**Figure 10,B**).

The first surprise was that as soon as there was a majority (even if it was only 60%, which does not seem so easy to detect) the females learned to prefer males of the colour chosen by the majority during the demonstration. In effect, all bars in **Figure 10,B** with a majority differ significantly from a random choice (the horizontal dashed line). Thus females were able to determine the majority and conformed to it. This seems quite surprising given the smallness of their brains and the complexity of the task.

The second surprise was that the four experimental treatments showing a majority in favour of the same colour did not differ significantly from each other. Thus females learned with similar efficiency to prefer the most commonly chosen male colour, regardless of the level of majority. It is a bit like students learning that two plus two makes four but occasionally (up to 40% of the time) hearing that two plus two makes five. What would you expect them to learn under such circumstances?

Figure 10: Drosophila females conform in love. A) The hexagon used to test conformity in mate copying. **B)** The results of the study in Drosophila[253]. The Y axis represent the proportion of observer females copulating with the pink male. The dotted horizontal line at 0.5 visualises what would be expected if observer females mated randomly. Values above this line indicate that the observer females showed a tendency to copulate with the pink male and below this line they tended to prefer the green males. The numbers in the bars represent the sample sizes tested in each experimental treatment. P-values above bars indicate the probability of being wrong in stating that the outcome differs from random mating. The probability above the horizontal bars is the probability of being wrong in stating that the bars being compared differ from each other.



The Fisher runaway process

However, the comparison I just made is not entirely correct, because there are good evolutionary reasons for females to conform in the context of mate choice, which would not be the case in the addition example. Imagine a young female reaching reproductive age and looking for a mate. From an evolutionary standpoint, this is one of the most important choices in her life, as it will influence the quality and number of her offspring. There are therefore strong selection pressures acting on all individuals, and particularly on females, to be fussy when choosing a mate in general, and especially so in *Drosophila* where females have only few mating opportunities in nature.

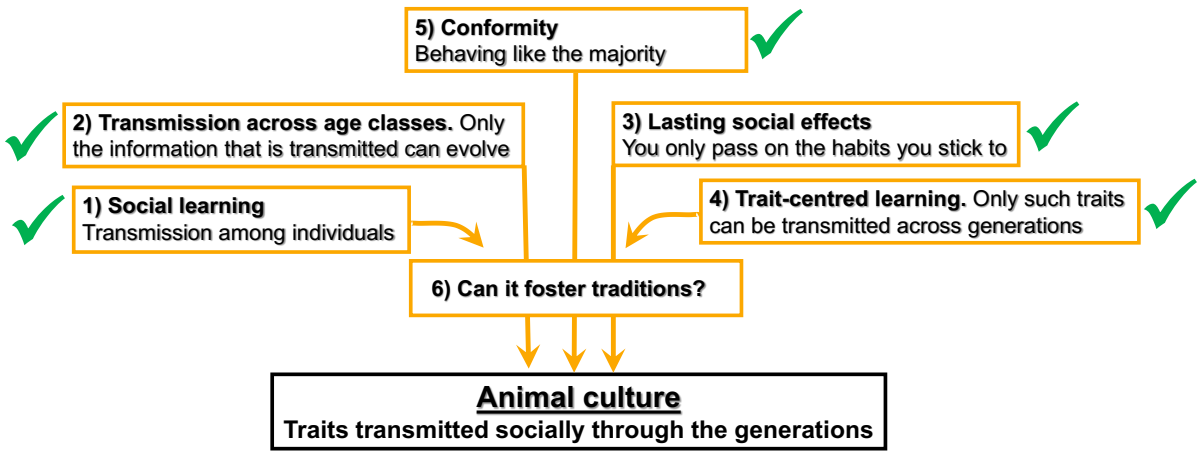
Our young female does not know which males to choose. Let's imagine that she mates with a male of the non-preferred phenotype in her population. Her sons will most likely inherit their father's phenotype and will therefore be non-preferred and have less access to females and therefore fewer offspring. So will her grandsons, and so on. It therefore appears that females who choose to mate with the locally non-preferred male phenotype (i.e. non-conformist females) are strongly counter-selected. Selection therefore favours high conformity in mate choice, which explains our surprising result. As a matter of facts, any female able to choose to have her offspring sired by a male of the locally preferred phenotype have a major evolutionary advantage. Thus, we should not have been surprised by this result. Our astonishment could also be seen as a lack of confidence in the power of natural selection, which is the last straw for an evolutionary biologist!

In fact, we could even have predicted conformity, especially since in 1930 *Ronald Fisher* described a similar phenomenon called the 'Fisher's runaway process'[254]. However, Fisher's process is not entirely equivalent to the one we are discussing here. Fisher reasoned within a homogeneous and isolated population. In such a case the end point is not the evolution of conformity but rather the transitory evolution of a preference for a given type of male leading to the disappearance of other types of males. This is how we explain the evolution of the bright colours of birds or of all the ornaments of the males of many species, such as the tail of peacocks or the antlers of deer. In order for conformity to evolve, the process must unfold within a metapopulation, i.e. a set of subpopulations connected by dispersal. The Fisher runaway process takes place in each subpopulation, and it is the fact that some individuals disperse among subpopulations that fosters the emergence of conformity[255]. It is therefore a little more complex than the Fisher process alone.

Are these five criteria sufficient for cultural traditions to emerge?

Thus, mate copying in Drosophila meets the five conditions identified in the literature as necessary for the emergence of long-lasting traditions (Figure 11). However, it remained to be verified that these measured properties can lead to the emergence of a population-wide preference for a given male, which we call tradition. To do this, we conducted an experimental transmission chain using hexagons, coupled with a mathematical model reproducing the conditions of this transmission chain[256].

Figure 11: A definition of culture centred on the properties of social learning. For a trait to be culturally transmitted across generations, it must meet five experimentally testable criteria. Furthermore, it is important to check that with the parameters measured during the testing of these criteria, this social learning can lead to the emergence of persistent local traditions in the specific case study. The green ticks added next to each criterion means that each of them has been shown to be met in the Drosophila study.



- In such transmission chains, the six observer females (or 'students') of one step of the chain become the six demonstrator females (or 'teachers') of the next transmission step, and so on, as in a kind of 'ear-to-ear' game in *Drosophila*. The 36 such transmission chains we performed lasted much longer than predicted by chance:

the observed number of transmission chains that reached the eighth step without a change of majority was 142 times higher than predicted by chance.

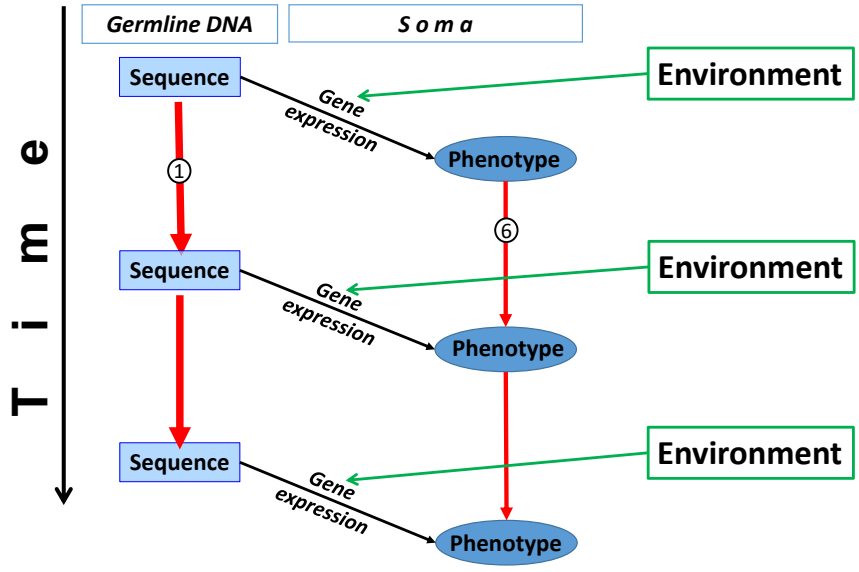
- We also found that the mathematical model developed by *Arnaud Pocheville* reproduced our experimental results very accurately.
- Based on this experimental validation of the model, we explored the extent to which mate copying can foster the emergence of long-lasting collective preferences for a given male phenotype, on a population scale, i.e. the extent to which this social learning can lead to the emergence of local traditions that persist across generations. This analysis showed that conformity was essential for the emergence of traditions, and that with certain parameter sets we could observe the emergence of traditions that persisted for at least 100,000 transmission steps, which would correspond to over 9,000 *Drosophila* generations.
- It turns out that drosophila have all the cognitive abilities to transmit their sexual preferences culturally potentially giving rise to traditions persisting over many generations. All this contradicted my initial assertion that it was impossible for *Drosophila* to have cultural processes. Our intuitions are sometimes very wrong.

Strengths and weaknesses of our approach

The main weakness of the *Drosophila* study, as well as other studies of cultural processes in insects[257], is that while it demonstrates in the laboratory that drosophila *can* transmit certain traits culturally, it does not demonstrate that they *do* use this capacity in nature. In this sense, this type of study in insects has the mirror-image flaws of vertebrate studies describing the existence of traditions in nature. In vertebrates, there are many examples of persistent behavioural variation suggesting a cultural phenomenon in nature (if these traditions are indeed the result of social learning, which remains to be demonstrated in many cases). Contrastingly, in insects we are beginning to have strong arguments about the ability to learn from others in a way that can foster traditions, but we do not yet have any examples showing that such local traditions exist in nature. There is therefore a strong complementarity between these approaches. Another weakness of the *Drosophila* study is that it uses artificial and contrasting male phenotypes. It will be interesting to explore what happens with more natural traits[258] and with more than two phenotypes[259].

An undeniable strength of our approach (as well as of several recent studies[260]) is that it is experimental. It proposes and applies a general definition of culture based on experimentally testable criteria, providing a kind of "toolbox" that can be transposed to a large number of species. Only an approach of this type in a large number of species will make it possible to begin to document the taxonomic scope of animal culture and thus its role in evolution. Another strength is that this approach focuses on the main features of social learning for it to lead to cultural traditions, including conformity that although rather well studied in humans remains to be explored in the rest of the animal kingdom[261]. For this, it will be necessary to manipulate the level of majority in the experimental population, an important condition for making progress.

Figure 12: **The intergenerational information pathway generated by cultural transmission**. According to this pathway (arrow 6) social learning generates vertical across generations information transfer from the parents' phenotype to that of their offspring[262]. This diagram, however, visualises only one of the fundamental aspects of cultural transmission (see Figure 13).



A general definition of culture applicable to any species

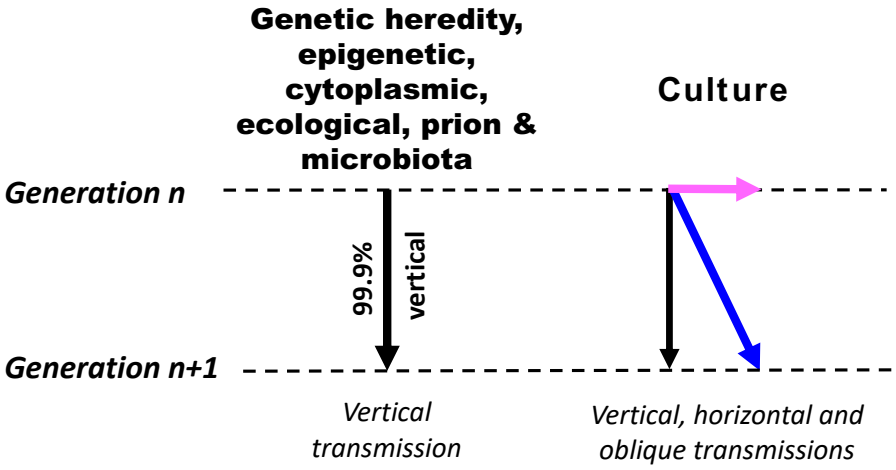
We can now propose a basic definition of culture that is applicable to any animal species including humans. *Animal culture is that part of phenotypic variation that is inherited through (1) a form of social learning, that (2) occurs regularly from older to younger individuals, (3) is memorized for long enough to be copied, is (4) centred on traits (and not on individuals), and (5) incorporates a process of repair or reinforcement (such as conformity, or the existence of a punishment system, or digitization) that allow alternatives to be maintained at a low frequency (Figure 11). (6)* We can also add a sixth criterion, which is to *check, for example through a theoretical approach, that collectively these five conditions have the capacity to give rise to persistent local traditions, which is the main marker of culture.* It is the sixth condition of this definition that links it to the previous definitions that mainly focused on persistent patterns of behavioural variation among populations.

A new inheritance pathway

Before concluding this chapter, we need to return to the diagram of information flows across generations as introduced in **Erreur ! Source du renvoi introuvable.** We can now add a new pathway of intergenerational transmission (arrow 6 in Figure 12).

Figure 12 may suggest that the properties of the cultural and the genetic pathways are very similar, but this is a misleading impression (Figure 13). As a matter of facts, whereas genetic transmission, as well as all the other forms of transmission we have seen in previous chapters, are essentially vertical (i.e. from parents to offspring[263]), cultural transmission is unique in that it also has strong horizontal (between individuals of the same age from different family lines) or oblique (between unrelated individuals of different generations) components. This highly originality is likely to profoundly affect evolution.

Figure 13: Originalities of cultural transmission[264]. Cultural transmission is unique in that, unlike all other forms of inheritance, it can be transmitted horizontally and obliquely. As theoretical approaches have shown, this particularity has major consequences for evolutionary dynamics. However, it should be pointed out that horizontal gene transfer can occur, although extremely rarely, in eukaryotes. This is why it is stated that 99.9% of all other forms of transmission are purely vertical. The number 99.9% is just a formulation to state that these other forms of transmission are almost exclusively vertical, i.e. from parents to offspring, but that there are very rare exceptions.



Conclusions

It is now time to conclude on cultural transmission. I have taken the time to detail this process for various reasons, which are summarised below.

- *Animal culture must be thought of as a process of heredity*. One of the central messages of this chapter is that social learning, and the cultural process that can result from it, generate a transfer of information across generations leading to parent-offspring resemblance. Thus, behaviour is not only a process of adaptations to rapid changes in the environment, as is widely believed[265], but also intrinsically an inheritance process.
- *Learning can take two forms*. At the end of Chapter 8 we saw that the widely accepted idea that there are only two possible alternatives to environmental change, genetic engraving vs. learning in the environment, is not correct in view of the existence of many other forms of non-genetic inheritance. However, the existence of cultural inheritance shows that the learning strategy needs to be split into individual *versus* social learning. Individual learning effectively leads each individual to relearn its environment in real time, thus offering high reversibility. However, as we have just seen, social learning can potentially lead to much more stable dynamics by generating very long-lasting traditions that can perhaps persist for thousands of generations[266].

- *We must study the cultural process also in non-human animals*. Although the literature on culture has been and still is dominated by human sciences, it is of prime importance to develop the study of animal culture as such if we are to understand the evolutionary origin of human culture with all its relatively recent evolutionary consequences for our species.
- *Originalities of cultural inheritance*. Cultural transmission comprises a highly original component of parent-offspring resemblance, both because it relies on sophisticated processes integrating information about the environment —in particular the social environment— and because it has unique transmission properties compared to all other inheritance processes identified to date (**Figure 13**). In this sense, the cultural process stands out from all other inheritance systems, which suggests that it may play a very special role in the evolution.
- *An inclusive definition of heredity*. The classic definition of heredity (see **Chapter 1**) only include resemblance resulting from vertical transmission, i.e. from parent to offspring and hence among relatives. However, non-vertical transmission also exist, in genes (though very rarely in eukaryotes but more commonly in prokaryotes), and recurrently in cultural inheritance as we saw, hence fostering resemblance among non-relative members of the same population (i.e. a group of interacting individuals). A more general definition of heredity could thus be "*patterns of resemblance that result from the transmission of some information among individuals*". From here on, I will thus use the phrase "*transmitted resemblance*" as a short of this new definition. Information here is understood in a broad meaning. It includes information with a well-defined avatar, (*i*) as in genetic (DNA sequence), (*ii*) epigenetic state (epigenetic marks), or (*iii*) in the form of a stable molecule shape and function (prions and chaperon). It also includes information with avatars that are less easy to define, such as (*iv*) the transmission of stable cellular states beyond epigenetic states, or (*v*) that of the stable environmental state, or (*vi*) the transmission of microbiota. Finally, it also includes (*vii*) information transmitted among individuals that has no real avatar as through social learning and cultural inheritance. As we saw in **Chapter 1**, *the important point is that such transmission should lead to resemblance that is stable intergenerationally.*
- *Cultural inheritance interacts with sexual selection*. All models of sexual selection assume that (*i*) female sexual preferences are encoded in their genes, and (*ii*) that females choose independently from each other. These two assumptions are clearly challenged by the existence of mate copying. Mate copying shows that often sexual preferences are learned early in life by observing the choices of older conspecifics, thus paving the way for the social runaway that we call culture. On the other hand, mate copying also shows that female choices are not independent, as females copy each other, which, through the process of collective runaway described above, can lead to strong local collective preferences, which must then strongly amplify the sexual selection exerted by females on male traits.
- *Animal culture can affect morphological traits*. Contrary to what is often thought, the transmitted resemblance generated by cultural transmission does not only concern the transmission of behaviour (language, or various habits), but can also affect morphological traits. For example, what is more culturally inheritable than dietary habits? These same habits strongly affect morphology, health, resistance to effort and, beyond that, phenotypic fitness. Cultural inheritance can thus concern resemblance on many phenotypic traits beyond behaviour.
- *Cultural inheritance is probably everywhere*. The *Drosophila* study suggests that the cultural process exists in an invertebrate not known for its cognitive and social skills. If this ability to culturally transmit such important traits as sexual preferences is indeed expressed in nature (which, as we have seen, has yet to be demonstrated), this would suggest that the cultural process is likely to exist in a very broad spectrum of species belonging to very diverse groups of animals. This would imply that it is necessary to incorporate its particular transmission properties into the study of evolution.
- *The frequency of culturally transmitted traits*. I expect many discoveries concerning the taxonomic range of animal culture. In effect, if one thinks about it, both the case of the missing heritability (see Erreur ! Source du renvoi introuvable.), as well as the whole range of non-genetic inheritance processes developed in this second part, point to a much more massive importance of non-genetic inheritance than is generally thought. There are many cases where authors first claimed that the fact that a trait is heritable reveals some genetic (i.e. sequencic) variation, and that were later shown to be non-genetically inherited. For example, the ability of twin species of Cichlid fish from Lake Vitoria in central Africa (*Pundamilia pundamilia* and *P. nyererei*) to avoid hybridisation was first attributed to genes controlling female preferences[267]. However, less than two years later another study using early in life cross-fostering experiments showed that this heredity in fact results from early life social imprinting for the parents who cared for the fry[268]. In fact, the fry learns to recognise members of their species by looking at the adults who care for them early in life and, for the rest of their lives, they seek to mate with individuals that resemble those who raised them. Thus, if the clutch of one species is cross-fostered with a pair of the other species, the resulting adults seek to mate with individuals of the other species. It is what was demonstrated in the second article. It was not gene based heritability but

rather social inheritance. This case shows how dangerous it is to use only the transmission patterns to infer the underlying mechanisms of inheritance. Because of the similarity of the patterns of variation generated by genetic and non-genetic inheritance, I usually say in a provocative way that *nothing looks more like genetic inheritance than non-genetic inheritance*.

- *A pioneering approach now lagging behind*. Although the study of culture was a pioneer in showing that the purely sequencic view of heredity was insufficient to explain the complexity of life, it is striking that today this scientific domain is still in its infancy. There are many reasons for this. The fact that we tend to think that cultural processes are unique to humans is one of them. The lack of experimental approaches is another, as well as the lack of a general definition applicable to any organism. Clearly, the time is ripe to overcome our prejudices and take a more integrated approach. In fact, the anthropocentric view of culture has prevented us from developing concepts and tools suitable for animal research. In particular, we lacked a definition of culture that went beyond the mere description of patterns of behavioural variation among populations.
- *Not molecular, therefore not serious*. Another element of context is that since the 1970s, many biologists consider that a process is only demonstrated once the underlying molecular mechanisms are known. For them, it is the knowledge of these molecular mechanisms that sanctions the scientific truth of a process. However, it should be noted here that this is an incorrect view of the way in which biological sciences often develop. Beyond the fact that this attitude denies the relevance of the four basic Tinbergen's approaches, it should be remembered that all the fundamental rules of genetics were developed in the first half of the 20th century, well before the discovery of DNA and its memory properties in the 1950s. In other words, this science flourished for 50 years in the absence of any knowledge about the underlying molecular mechanisms. And yet, all the major principles of genetics laid down during that early period are still valid today. The recurrent belief that I have been confronted with that only approaches incorporating molecular mechanisms are serious is therefore unfounded.
- *A bright future*. The final important message of this chapter is that the field of animal cultural evolution is an almost virgin continent to be fully explored, and that rapid progress must be made in this area, for otherwise this entire component of inheritance risks being completely ignored by the current modernisation of the Modern Synthesis of Evolution, a subject to which we shall return in **Chapter 21**. The question of the taxonomic range of the cultural process in the animal kingdom remains entirely unexplored. I would not be surprised if one day we discover very similar phenomena in plants, for instance. Furthermore, the uncovering of this taxonomic range will inform us about the age of cultural transmission in the evolutionary history of life. This highly desirable exploration will require experimental approaches to begin to unravel the complex issue of causality. It will also, of course, be necessary to study the neurobiological basis of social learning and the long-term memory of socially learned behaviour. This is, among other things, what we are currently working on in the fruit fly under the leadership of *Guillaume Isabel*, a specialist in the neuro-genetics of memory in this species.

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It will also be important to determine the extent to which this type of inheritance might change the overall evolutionary functioning, which will involve further development of theoretical approaches. My hunch, based on the theoretical approaches developed in humans, is that this may lead populations to *trajectories* (see Glossary) that would not be achievable with genetic inheritance alone.

Part Three

The Inclusive Evolutionary Synthesis

In the first part of this book I described some of the general principles of the Modern Synthesis of Evolution as I had been taught and as I taught it in unison with my colleagues until about twenty years ago. In the second part I detailed a selection of the arguments revealing the limitations of that synthesis. However, we will see briefly in Chapter 15 other domains of biology that provide important additional arguments. The main message is that we now need to integrate all forms of non-genetic inheritance documented to date into a new evolutionary synthesis that generalises the Modern Synthesis of Evolution.

The purpose of this third part is to integrate all these facts into a new *inclusive* framework. It is this new framework that I have been calling the *Inclusive Evolutionary Synthesis* (or IES)[269] since 2013. In doing so, one of my aims is to convince you of the need to use the term 'inclusive' rather than just the term 'extended' to name that new framework as many of my colleagues currently do in calling it the 'Extended Evolutionary Synthesis'. We shall see that what differentiates these two emerging perspectives lies essentially in the ambition to achieve the comprehensiveness necessary to integrate all known pathways of inheritance into a single synthetic theory.

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Chapter 12
The Modern Synthesis of Evolution is not incorrect, it is just incomplete

In March 2014 I was invited to speak about inclusive inheritance in a symposium entitled "Evolution: the variable genome of humans" at the Congress of the German Society for Human Genetics taking place in Essen, Germany. The discussion after my talk was monopolised by one person who literally blasted me by saying that the question of the missing heritability had long been solved —the proof being that there was none in cows— and by accusing me of putting Lamarck back on the map. He had spoken so much that I had no time to respond. I am used to getting hostile reactions to my talks. After all, as *Axel Meyer*, a professor of evolutionary biology at the University of Konstanz, told me after my talk, my presentations can be seen as challenging all the certitudes of a part of the audience[270]. A classic way out in such situations is to attack head-on in order to safeguard the current model, forgetting that it is only a simplification of reality. This is what this person did in 2014.

Moreover, the arguments used by that person to discredit my view were very weak. Indeed, there are two types of counterexamples, those that disprove the rule and those that confirm it, and the case of cows clearly fell into the second category because they are so inbred and modified by millennia of human domestication and manipulation that they are anything but representative of what happens in nature. Moreover, at the time, I never mentioned Lamarck. Since then, I have learned to acknowledge that there are some Lamarckian-like processes in inheritance. The sole purpose of my interlocutor's statement was to question the reality of the existence of various non-genetic processes of transmitted resemblance. Again, in science, facts must have the last word. The reaction to facts that are incompatible with our current conceptions should not be to reject the facts but, on the contrary, once these facts are duly established, to revise our conceptions so that our new conception is compatible with these facts. As *Karl Popper* clearly stated, this is precisely how knowledge advances. Furthermore, rejecting well-documented facts boils down to turning the current model into a dogma, i.e. an a priori belief, which is tantamount to leaving the domain of science.

Of course, I am not the only one to experience this kind of reaction. A good example is that of *Michael Skinner*, whose woes were even the subject of an article entitled "The Epigenetics Heretic" in *Science* in 2014[271]. This article recounts *Michael Skinner*'s setbacks following the publications we outlined in **Chapter 7**. To cut a long story short, this maverick researcher, who was among the first to show that responses to environmental stresses can be passed on to subsequent generations, was ostracised from getting funding to continue his research. And yet, as we have seen in detail, numerous studies carried out by other teams have since revealed sophisticated molecular mechanisms that confirm that certain responses to environmental stresses can indeed be passed on through gametes over many generations.

These examples are just a sample of the hostility that can be faced when defending the need to take into account non-genetic inheritance in biology in general and evolutionary biology in particular. This chapter aims to answer such recurrent commentaries.

Lamarck revisited

Ever since I started studying biology I have heard that Lamarck's view was wrong, even ridiculous. However, if one takes the time to read what he wrote, one realises that his ideas have often been distorted and caricatured in an unwarranted way. First of all, what Lamarck advocated was absolutely central to his time because he was the first to really formalise the idea that species change over generations. We saw in Erreur ! Source du renvoi introuvable. that at the time this was called transformism, which is the equivalent of what we mean by evolution today. Of course, Lamarck proposed mechanisms to explain these patterns of change, in particular what we now call "heredity through use and non-use", on the one hand, and "heredity of acquired characters" on the other.

Today, the so-called Lamarckism includes the idea that an organism can transmit to its progeny certain physical characteristics that it has developed during its own life. This idea is often referred to pejoratively as ‘soft’ inheritance, as opposed to ‘hard’ inheritance.

The classic example is that of the giraffe's neck, which, according to this view, would have lengthened over generations because, by pulling on it during their lives to reach the high branches of trees, giraffe parents would have transmitted a slightly longer neck than theirs. This idea would lead, for example, to children of very athletic parents to show a natural tendency to perform better in the sports practised by their parents, *only because their parents actually practised it a lot*, and not because they had inheritable intrinsic skills.

It is Lamarck's caricature that has been invalidated

Historically, this view, which is now called Lamarckism, has in fact been discredited with very bad arguments. First of all, the attribution of this idea to Lamarck is abusive because this vision of heredity was the dominant one among scientists at the time. It is therefore a bit abusive to attribute it to Lamarck alone. It is even more incorrect to contrast Lamarck with Darwin as people often do, because Darwin some 50 years later fully embraced a view of heredity that implied the inheritance of acquired traits. We shall see in **Chapter 16** that he had even formalised this idea with “pangenesis”, which, as we realise today, closely resembles some aspects of non-genetic inheritance.

Figure 14: An image published in 1832 that is regularly used to illustrate, and in fact ridicule, Lamarck's ideas[272]. The English expression "A chip off the old block" is used to refer to someone who is very similar in character to his or her father or mother, and who is a carbon copy of his or her parents.

Secondly, Lamarck's ideas have been widely caricatured, for example by using images such as the one in **Figure 14**, which is used to illustrate the common expression 'A chip of the old block' to mean something like 'like father, like son'. Historically, August Weismann's experiment, which is considered to have invalidated Lamarck's ideas, is a cartoonish distortion of the same kind as the one shown in this figure. The experiment consisted of cutting off the tails of mice over several generations before they reached breeding age. Having observed that after several generations the tail did not tend to disappear, he concluded that Lamarck was wrong. But, this is no more than attacking a strawman, and in effect, it is caricatures such as the one presented in **Figure 14** that have been invalidated, not Lamarck's ideas.

As a result of this experience, which is pure caricature, all the facts that seem to support the idea that individuals can transmit traits developed during their lives have been systematically rejected either as incorrect[273], as frauds or genetic contaminations, or at best as marginal. In fact, whenever a trait is transmitted, it is inevitably inferred that this transmission is genetic in nature, while being careful not to specify what meaning is given to the word genetic, thus maintaining the ambiguity we discussed in **Heredity concerns** patterns of parent-offspring resemblance. It is central to biology because natural selection and evolution cannot occur without heredity. It is thus vital to study the mechanisms that produce this resemblance that involves the transmission of many kinds of information from parents to offspring. Living organisms can therefore be defined as a 'memory machine' able to collect, store, use and then transmit a wide variety of environmental information. The study of heredity is therefore the study of the different forms of information that can be transmitted across generations and affect parent-offspring resemblance. However, during the 20th century, due to the fantastic discovery of the DNA molecule and its incredible sequencic memory properties, we became blind to the existence of other types of transmission mechanisms. As a result, we have increasingly reduced heredity to its sequencic component, i.e. the sole transfer of the information encoded into the nucleotidic

sequence of DNA, an attitude that I call sequencic. It is now time to re-open our views of inheritance to approach it in all its complexity. A first step to achieve this goal is to reflect about the gene concept.

Chapter 2

However, at the beginning of the third millennium, our ability to study the sequencic avatar of heritable information grew so efficient that it became clear that sequencic information was not sufficient to explain all the complexity of life, as illustrated by missing heritability (see Erreur ! Source du renvoi introuvable.). More generally, all the facts reported in the second part of this book force us to rethink these ideas and to admit that certain characters developed during the life of individuals can actually be transmitted to the progeny. This is an indisputable fact that we must take on board.

No, in the end, everything does not boils down to genes

The concept of emergent property

A recurring comment after my talks can be summed up as "Yes, but in the end it all comes down to genes". Following my excellent colleague *Lounès Chikhi* in Toulouse, I usually answer this: "Indeed, everything in biology is based on genes. But if you think about it, genes are themselves made up of atoms, which are composed of neutrons, protons and electrons, and if you break them down even further, you end up with quarks, which are the basic components of all the matter around us. So, if I follow your reasoning to the end, I can say 'Yes, but in the end it all comes down to quarks, so let's only study quarks' ".

In fact, the claim that everything is based on genes ignores the important concept of "emergent property"[274] according to which *the properties of an aggregate of many entities are more than the sum of the properties of its component entities*. Something emerges at the scale of the aggregate that is of a different nature from that of the component entities. This implies that in order to study the properties of the whole, one must use other approaches, theories and concepts than those used to study the components[275]. A telling example is that of the thought that emerges from the functioning of some 90 billion neurons and 10,000 billion synapses per cm^3 in our brain. Using only neurological approaches (with electrodes planted in the brain to look for the activity of this or that neuron, for example) will probably never enable us to understand thought because it is an emergent property of the brain. To study thought, we need to use other approaches, such as psychology or philosophy, which really have little to do with neurological approaches.

The concept of emergent properties is at the very heart of biology. One could say that life itself is an emergent property of the components of organisms. At each level along the scale of complexity and interaction, from molecules, organelles, cells, tissues, organs, individuals, populations and ecosystems, and even the biosphere, one or more new sets of properties emerge that cannot be explained by the properties of the lower level alone. It is even possible to trace a history of the appearance of major emergent properties that have punctuated the history of life. This is what *John Maynard Smith* and *Eors Szathmàry* did when they spoke of the major transitions in evolution[276].

Thus, just as it would be wrong to claim that we should study only quarks to understand the universe, it would be counterproductive to claim that the study of genes alone is sufficient to explain life, and the deep reason for this error is that it would not allow us to capture the effects of the stacking over genes of emergences of new properties that are of a completely different nature from those of the genes themselves. Of course, behind all non-genetic inheritance mechanisms we have described in this book, there is undeniable genetic support, but to claim that we can understand everything by studying genes alone would be as wrong as claiming that we can understand everything by studying quarks alone.

Taking the example of animal culture, the genetic information produces the template, essentially in the form of learning ability, by which behaviour develops and thus can vary according to all information gathered during development. But once learning ability has evolved because it provided a selective advantage in a given context, the social component of inheritance that we have defined as culture becomes a component of inclusive inheritance[277]. We have seen above that social learning exists in a vast array of animal species. Once operational, social learning unleashes behaviour from its underlying genes. Another formulation would be to say that genes, through their functioning in interaction with the environment, bring out properties that escape them completely. This is exactly the case with non-genetic inheritance. The Modern Synthesis of Evolution has somehow stopped at the level of the genetic replicator, probably because of its direct avatar in the form of the DNA sequence.

In this book, I push the logic initiated in Chapter 11 of The Selfish Gene[278], by integrating the various processes that may be called "*pseudo-replicators*" (see Glossary) emerging at all levels of organisation of living entities. From here on, I will use the term "*replicator*" (see Glossary) for genes only, and the term "pseudo-replicator" for entities that can replicate with a level of fidelity lower than that of the sequencic replicator. I will also use the term "*replicating entities*" (see Glossary) to encompass both the genetic replicator and all potential pseudo-replicators. The difference between these terms lays in the durability of the variants;

true replicators encompass variants that have indefinitely persistent fidelity, even if that fidelity is imperfect. Pseudo-replicators having a lower fidelity produce variants that can persist over much smaller numbers of generations. We will, however, see below in this chapter that the durability of the cultural and epigenetic variants may be far more long-lasting than usually envisioned.

The issue of the fidelity of transmission

Another common objection is that the fidelity of the various forms of non-genetic inheritance is too low to be able to influence evolution significantly. There are two errors in this statement.

First, it is unfounded to claim that the fidelity of non-genetic transmission is always low. Studies have shown that the rate of change of some epigenetic marks can be of the order of 10^{-4}, or one change per mark on average every ten thousand generations[279]. Amusingly, this is exactly the value I was taught for mutation rates in the 1970s, when sequencing was not available so that we only used detectable changes in phenotype. Since such phenotypic changes could just as well be due to persistent changes in the epigenetic state of certain genes, in fact that method mostly detected the effects of the most frequent sources of change, i.e. epigenetic change. The similarity between these two values is therefore probably not coincidental. Similarly, we saw in **Chapter 10**

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Randomness and mutation

After discovering all these fascinating pathways of intergenerational information transfer, it is now necessary to develop an overlooked but basic property of epigenetic marks that is linked to a recurring issue in evolutionary biology, namely that of the randomness of mutations of all types. We have seen that one of the basic principles of the Modern Synthesis is that mutations are in no way directed by the environment towards improving the adaptation of organisms. Unfortunately, this principle is often simplified into saying that mutations occur at random, which does not mean the same thing. But what exactly is the case? This is what we will look at in this chapter.

Epigenetic marks are mutagenic...

The starting point that led me to think about the issue of mutation randomness was the fact that epigenetic marks, such as the presence of methyl radicals on cytosines, destabilises DNA and greatly increases the mutation rate of methyl-cytosines into thymine, another base of the DNA sequence. This, therefore, has the potential to generate point mutations whereby a cytosine is replaced by a thymine. Some articles have, for example, subheadings entitled "Methylation is mutagenic". For example, studies in humans suggest that cytosine methylation is responsible for 30-40% of point mutations in the human germline. Combining the results of several authors, cytosine methylation would increase the probability of cytosine mutating to thymine by a factor of about 20,000. This is such a considerable factor that it seems very unlikely that it is a negative collateral effect of a process selected in another context (in this case DNA methylation, which is involved in the regulation of gene expression). What then could be the function of a process that destabilises the fidelity of sequencic transmission to such an extent?

This is what we addressed in a 2019 paper. We proposed a mechanism by which such mutagenic power of DNA methylation, and more generally of epigenetic marks, might have provided a real evolutionary advantage by accelerating the sequencic engraving of the initially plastic responses to environmental conditions that prove to be very persistent. We have given this mechanism the explicit but unmemorable name of *epigenetically-facilitated mutational assimilation*.

Genetic assimilation

The idea of *genetic assimilation* (see Glossary) was proposed by *Conrad Waddington* following a series of experiments in *Drosophila* showing that following an environmental stress triggering an initially plastic response, this response tends to become heritable (and therefore non-plastic) after a certain number of generations under the effect of this stress. It was therefore as if, after a few dozen generations, characters initially developed in a plastic manner in response to a given environment became ‘genetically’ engraved, hence the expression 'genetic assimilation'.

Genetic or epigenetic assimilation?

However, it should be noted that in this expression the term genetic was understood in its pre-DNA sense, as 'that which is transmitted', without prejudging the mechanism responsible for this transmission. In particular, while Waddington's experiments undoubtedly demonstrated that the initially plastic trait became inclusively heritable, they did not at all show that this necessarily implied a sequencic change. In effect, there was nothing in these experiments to suggest that what he observed at the phenotypic level resulted from a change in the DNA sequence. Given that Waddington had only worked over a few dozen generations —which was already a real challenge —he in fact most likely documented an "epigenetic assimilation" because the only thing his

experiments really showed was that an initially plastic trait became inclusively inheritable within a few generations. This is equivalent to what *Mary Jane West-Eberhard* called "genetic accommodation" whereby a trait can be made heritable without necessarily involving encoding in the DNA sequence. Our paper proposed that, under certain conditions to which we will return later in this chapter, this process could go as far as sequencic engraving, *if the environmental stress persists over many, many generations*.

And the Modern Synthesis assimilated genetic assimilation

It has always puzzled me that the idea of genetic assimilation has finally been 'assimilated' by the Modern Synthesis, as this mechanism is strongly reminiscent of the much-rejected idea of inheritance of acquired traits. If you think about it, Waddington's mechanism proposes that within a few dozen generations under a given constant environmental stress the initially plastic response to stress can become heritable. In fact, what has allowed the idea of genetic assimilation to be assimilated is the relative slowness of this phenomenon. Moreover, the classical interpretation of this phenomenon is that there would pre-exist some neutral and hidden sequencic variation (usually called standing genetic variation) that would be somehow revealed by the environmental stress. Natural selection would then have the time to act over the few dozen generations of Waddington's experiments to retain only those variants that happen to be, I would like to say 'miraculously', favourable. So genetic assimilation would be just a special case of natural selection. This is how the Modern Synthesis has managed to see no major contradiction in genetic assimilation. This is also how I understood it until a few years ago.

Epigenetics as a hub towards sequencic engraving

A striking result on which we have built our reasoning is that all mechanisms of non-genetic heritability seem to involve some epigenetic change. It is as if epigenetics was the backbone or hub towards which most non-genetic inheritance processes would converge. Then, as epigenetic marks destabilize the DNA, over the course of many generations, this would generate sequencic variation *in the parts of the DNA concerned by the accommodation to the environmental change*. This would lead through natural selection acting on this newly produced variation, to sequencic engraving. In a way, epigenetics would be the conductor of the orchestra made up of all the genetic information. In effect, while it is very useful to have all the sequencic information (the recipe book), it is important to use it wisely. We shall see in **Chapter 16** that this epigenetic conductor is itself under the control of the brain.

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With *Arnaud Pocheville*, then based at the University of Sydney in Australia, we modelled this idea and were able to show that such a mechanism could accelerate the transfer of epigenetic encoding to sequencic encoding by a factor of the order of magnitude of the mutagenicity of the epigenetic marks, i.e. about 20,000 times. *This is what we called the epigenetically-facilitated mutational assimilation*.

But the story does not end there, as epigenetics interacts strongly with another major source of mutation, namely transposable elements.

... and interact with transposable elements

In parallel, we have been interested in another major phenomenon that can affect both the expression of certain genes and the appearance of mutations of all types. In fact, not only can the presence of epigenetic marks affect the stability of DNA, but epigenetic marks are themselves in strong interaction with the activity of transposable elements. Transposable elements are mobile DNA sequences discovered in maize by *Barbara McClintock* at the Cold Spring Harbor Laboratory on Long Island in the USA in the 1940s. This is one of the great genetic discoveries of the second half of the 20th century. There are a variety of transposable elements that differ, among other things, in the way they duplicate. Transposable elements exist in almost all living organisms. They seem to be able to invade the genome of an entire species through a process of colonisation from a local population, and can represent a large portion of the genome (about 15 to 22% in Drosophila, 40% of the genome in humans, and up to 90% in wheat). To give an idea of the prevalence of transposable elements, in humans, more than three million human sequences are derived from transposable elements, but only a few hundred of these have retained transposition capacity. The universality and mobility of transposable elements suggest that they play an important role in genome evolution and plasticity

The activity of transposable elements is under epigenetic control

The activity of transposable elements is strongly modulated by epigenetic processes (involving methylation, histone modifications or small RNAs) which are themselves affected by environmental factors. There are several hypotheses (not necessarily mutually exclusive) explaining the interaction between transposable elements and epigenetics. In particular, the targeting of epigenetic modifications to transposable elements could be a consequence of the *exaptation* (see Glossary) of transposable elements as platforms for chromatin modification, in which case the epigenetic regulation of transposable elements could be a consequence of

genome defence and regulation. As a result, environmental stresses can trigger transposition activity, either directly or through their effects on epigenetic marks associated with transposable elements. It can be said that in most cases the mobility of transposable elements is inhibited by epigenetic marks that block their replication. However, this targeting of epigenetic marks on transposable elements also affects, as if by ricochet, the genes close to these transposable elements —with which they become partners in a kind of "transposable-element-gene duo"—, thus affecting their expression level. Beyond their important mutational effects, by duplicating themselves in the genome, transposable elements can thus affect the general functioning of the genome, among other things by regulating and controlling the activity of genes in the neighbourhood of their insertion point. Thus transposable elements affect gene activity in three different ways.

- First, by attracting strong epigenetic marking around their insertion point, they affect the epigenetic marks, and therefore the expression, of the genes with which they are in duo. It should be noted that the epigenetic marks around transposable elements can be modified by stresses bringing back their mobility, hence modifying the expression of the genes around the new insertion point.
- On the other hand, as the sequence of many transposable elements carries regulatory elements of response to the environment, their presence will directly modulate the expression of the genes with which they are in duo according to the environmental context. They therefore play a central role in the response to environmental changes.
- Finally, by their mobility within the genome, transposable elements can generate significant sequencic changes in the genome. Their mutagenic potential is thought to increase the average point mutation rate by several tens of thousands of times.

A great generator of inclusively heritable variation

Thus, the presence of transposable elements in one area of the genome can on the one hand durably modify the expression of the surrounding genes due to the strong intervention of persistent epigenetic marks inhibiting their mobility, and on the other hand generate genetic (sequencic) variation in the whole genome as a result of their mobility. Both types of variation can affect the phenotype either negatively for individuals (e.g. they are implicated in various diseases) or positively at the population level by generating variation that is inclusively heritable and therefore open to selection. In other words, while at the individual level these changes can often have negative consequences, at the population level transposable elements generate inclusively heritable variation on which natural selection can act, thus favouring the adaptation of populations to their environment.

Interactions between epigenetics and transposable elements thus constitute a real engine for the creation of phenotypic variation (targeted to specific portions of the genome) that can be inherited either sequentially or epigenetically *in response to environmental stresses*, and are thus an important factor in evolution. Such a generator of genetic and epigenetic variation can in particular explain changes in mutability within the genome following environmental stresses. Several authors have emphasised the existence and importance of such generators of inclusively heritable variation involving the joint action of genetic and non-genetic processes in the ability of natural populations to adapt to ongoing global changes under the influence of human activities.

Epigenetically-facilitated mutational assimilation

We can now synthesize this. It appears that the effects of environmental stresses can affect the expression of specific genes involved in the response to stress and affect the activity of transposable elements, two major characteristics that each have the capacity to increase the sequencic mutation rate by tens of thousands of times, which is anything but negligible.

An information transfer pathway acting over many generations

The epigenetic changes affecting the expression of genes specifically involved in the response to an environmental stress in fact have two functions taking place on two very different time scale:

- First, these epigenetic marks, which we have seen target very precise portions of the DNA, enable the individual to adapt to the current environment by finely regulating the expression of the genes involved and leading to the phenotypic response to the environmental challenge. This response is rapidly established under the effect of environmental change. This process is known as phenotypic plasticity, the ability to modify the phenotype in response to the environment.
- Second, by being inherited, those epigenetic marks lastingly affect the mutability of the concerned genes that happen to be the genes involved in the accommodation to the specific environmental change. These epigenetic marks can also affect the activity of neighbouring transposable elements, which can further increase the mutability of the concerned regions and thus the potential generation of sequencic variation. In other words, epigenetic marking would differentially mark portions of the genome for mutation, i.e. for the generation of sequencic variation and thus for the multigenerational exploration of new genetic possibilities. Far from being a cost in terms of evolution, this may on the contrary constitute a major evolutionary benefit because the

sequencic variation thus generated concerns the genes actually involved in the accommodation to the specific environmental stress, a variation then open to natural selection.

This is *epigenetically-facilitated mutational assimilation* that is more than just a special case of natural selection on initially neutral and hidden genetic variation suddenly revealed by environmental change. According to our view, genetic assimilation appears as *a genuine mechanism for manufacturing sequencic variation in the parts of the genome concerned by the accommodation to the specific environment*, variation which is then open to natural selection. This mechanism calls for several important comments.

Random mutations in environmentally targeted areas of the genome

First, with epigenetically-facilitated mutational assimilation, the fundamental axiom of the Modern Synthesis that *mutations are not influenced by the environment in an adaptive direction* remains 100% valid. However, it is the simplified phrase traditionally used to simplify this axiom "mutations are random" that appears incorrect. With epigenetically-facilitated mutational assimilation the mutations generated following a lasting environmental change are indeed not influenced in an adaptive direction by the environment (the axiom of the Modern Synthesis therefore remains valid), but the parts of the genome where the mutation rate increases are actually targeted by the environment. *This is because epigenetic changes and the activity of transposable elements are themselves targeted by the environment*. There are therefore two independent scales where randomness can be expressed, that of regional portions of the DNA, and that of the local change of sequence itself. Only the second scale is unaffected by the environment, whereas the regional scale is clearly targeted by the effects of the environment in the sense that it is precisely in the portions of the DNA concerned by the accommodation to the environmental challenge that the mutation rate changes.

A necessarily slow process…

Second, even if the magnitude of several tens of thousands of increase in mutation rate seems enormous, it does not mean that epigenetically-facilitated mutational assimilation (i.e. the sequencic engraving of the adaptation) takes place in a few generations. A rough calculation predicts that such a process must take hundreds, if not thousands, of generations to become effective. Although the calculation proposed in the last note is very crude, the important point is that we should not expect epigenetically-facilitated mutational assimilation to take place very quickly, and certainly not in only a few tens of generations. And in fact, evolutionary logic even leads us to believe that this slowness is integral to the process (see below).

… which could be involved in domestication

We were certainly not the first to think about this type of genetic assimilation where the environment can be involved in generating genetic variation in the sections of the genome involved in the response to the environment. For example, one of the earliest papers on the subject dates back to 1983 in which *Hugh Iltis*, then Professor of Botany at the University of Wisconsin, formalised a scenario for the domestication of maize from teosinte, an annual plant from Central America. This remarkable scenario integrated several previous hypotheses and involved the major and massive effect of what he called a catastrophic epigenetic sexual transmutation that occurred some seven millennia ago.

Similarly, the whole literature on transposable elements claims that the environment can generate inclusively heritable variation. Regarding the idea that the environment can generate variation in certain regions of the genome, *Eva Jablonka* and her collaborators had modelled this idea without proposing a molecular mechanism. Similarly, *Michael Skinner* also foresaw and proposed the existence of such phenomena. Furthermore, researchers working on the domestication syndrome of vertebrates proposed that the stress induced at the beginning of domestication must have caused alterations in the methylation patterns of developmental genes expressed in the neural crest (the part of the embryo that will become the central nervous system), epigenetic changes that could have been fixed in the form of genetic variants to explain recurrent behavioural resemblances in the many domesticated fish, mammals and birds.

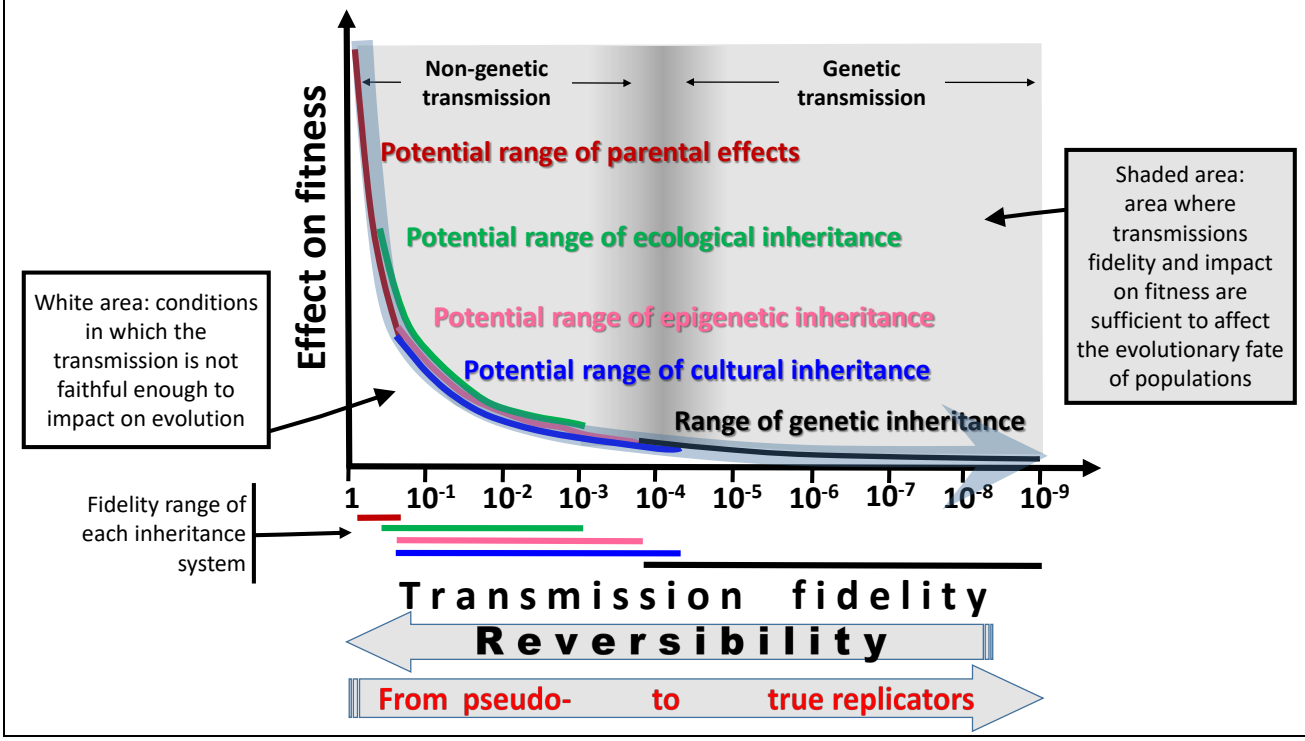
The different systems of inheritance interact with each other

This chapter thus introduced a particularly important point, namely that the different systems of inheritance (which we will summarise in **Chapter 15**) do not operate independently of each other. On the contrary, they interact and influence each other. For example, the central idea of epigenetically-facilitated mutational assimilation is that the molecular memory represented by epigenetics states interacts over the long term with sequencic memory, in a way that can potentially considerably accelerate the genetic encoding of initially plastic responses to environmental characteristics that persisted for hundreds or thousands of generations.

Chapter 11, that, according to some models, cultural transmission can show a remarkable durability of up to tens of thousands of generations[280]. While it is undeniable that the various forms of non-genetic transmission are less durable than sequencic information, the lability of non-genetic inheritance should not be exaggerated.

Second, we shall see in Chapter 16 that far from being a weakness, this relative lack of fidelity of the various non-genetic inheritance systems is a real strength. The evolutionary impact of an inheritance mechanism does not depend solely on its transmission fidelity (Figure 15). Information strongly affecting the fitness of individuals possessing it (e.g. an all-or-nothing effect such as survival or death) does not need to be transmitted over many generations to affect the fate of a population. Such all-or-nothing effects exist in nature. For example, in the black-legged kittiwake (*R. tridactyla*), there is a documented maternal effect whereby females confronted with Lyme disease deposit antibodies against the Lyme bacteria in the yolk of their eggs[281]. These antibodies make their chicks resistant to the bacteria during their stay in the nest, which is when the bacteria is transmitted to the chicks by ticks (*Ixodes uriae*) that often infest the nests of this species. In a population composed of females that do or do not transmit such antibodies, the offspring of the former would survive while those of latter would die from the disease. It is therefore a kind of all-or-nothing effect on fitness. The fate of the different family lines of this species must therefore be strongly influenced by the ability provided by this maternal effect to resist a parasite. Thus, although this maternal effect is very often only transmitted over a few generations (and is therefore said to have low fidelity), it nevertheless plays an important role in natural selection and thus in evolution, as is clearly illustrated in Figure 15.

Figure 15: **Relationship between transmission fidelity and the minimum effect needed for it to affect evolution**. Transmission fidelity on the X axis is represented by the logarithm of the point mutation rate per generation. It increases from left to right, whereas the reverse is true for reversibility. That axis also visualizes the switch from pseudo-replicators to true replicators. The Y axis represents the effect of variants on fitness, and hence the potential of selection to act on the concerned variant. This figure shows that the transmission of a trait across generations can give rise to evolution by natural selection either via high fidelity (leading it to remain unchanged over many generations) or via having a very strong effect on fitness. The stronger the effect on fitness, the lower the minimum fidelity needed for it to affect evolution. The shaded area represents the range of possibilities. Below this zone, information would be too labile to affect evolution effectively. The curve at the bottom of this area is broken down into several colours, each representing the plausible range of action of each inheritance mechanism in view of estimates reported in the literature. Projections of these curves onto the transmission fidelity axis give an idea of the window of plausible fidelities of that mode of transmission (coloured lines below the fidelity axis). For example, parental effects are usually assumed to be transmitted only over a small number of generations. This is the most labile type of transmission that cannot give rise to a true replicator. I thus call it pseudo-replicator to sanction the fact that they are heritable, but only over a small number of generations. Conversely, some estimates suggest that point mutations occur only very rarely up to once every billion generations. The actual values are probably more in the order of once in a million generations, so that genetic transmission is the most faithful. But as this graph shows, this certainly does not mean that only the inheritance of sequencic information can affect evolution. In particular, the large bluish arrow that runs from left to right at the bottom of the grey area underlines the fact that the encoding of heritable information can switch from labile to more and more stable avatars when the environmental stressor persists for more and more generation after its first appearance. Chapter 10 develops that idea. Thus the various systems of inheritance are active on very different timescales, which makes them particularly complementary. Finally, the vertical darker shaded area in the grey area marks the temporal boundary between selection for genetic (right) and non-genetic (left) encoding[282].



Thus, the argument that non-genetic transmission is too labile to affect evolution is not really relevant as we should also account for the size of the effect on fitness. Moreover, **Figure 15** shows that due to its very high transmission fidelity, a genetic variant providing a very small advantage can still be selected for over many generations, if conditions do not change in the meantime. This is why many traits are influenced by hundreds of different genes, most having a very small but real effect on the concerned trait.

Estimating the range of transmission fidelity of all non-genetic inheritance systems

As **Figure 15** underlines, we have some knowledge about the level of fidelity of the various inheritance systems. As we saw in **Chapter 11**, cultural inheritance can be very long lasting, potentially persisting over thousands or more generations[283]. This was sensed by *Richard Dawkins* when he explored in The Selfish Gene the possibility of the cultural process to lead to the emergence of another replicator. However, this still needs to be investigated further.

Concerning the epigenetic pseudo-replicator, a study in *Arabidopsis thaliana* showed that rates of change can be as low as 10^{-4} per CG pair and generation. For instance, talking of the methylation of CG pairs, these authors say "*We estimated a lower bound of the epimutation rate with the linear regression results, which revealed 4.46 x 10^{-4} methylation polymorphism per CG site per generation ($P < 0.0000216$)*"[284]. The fact that this estimate concerns a plant is particularly interesting because it is considered that environmental effects are far more likely to be transmitted to the offspring in plants than in animals, making this estimate conservative.

Thus, the high levels of transmission fidelity potentially reached by the cultural and epigenetic inheritance systems would certainly allow the resulting cultural traditions, or the epigenetic states (here engraved in the methylation of CG nucleotides) to qualify as "pseudo-replicators", if not replicators at all. Nonetheless, at this stage, I prefer calling them "pseudo-replicators" until further studies confirm their very high transmission fidelity in a series of model organisms.

A general message is that beyond these remarkable estimates of transmission fidelity of cultural and epigenetic inheritance, we still lack such estimates for most other inheritance systems. It is thus very important to get real estimates of transmission fidelity of all non-genetic inheritance systems to be able to define more precisely their range of action, because those presented below the X axis of **Figure 15** still remain partly speculative.

What plastic responses should be transmitted?

Another important question is which plastic responses should be transmitted and which should not. This is indeed a central issue[285]. It amounts to asking which plastic responses would benefit in terms of fitness to be passed on to offspring. This question is related to that of the different timescales of evolution that we addressed in **Figure 15**.

Each inheritance system operates with its own temporality

Figure 15 provides a visual approach to this fundamental issue. First, the extreme left part of this figure visualises why responses to environmental changes occurring more frequently than the time of one generation should not be passed on. Rather, in such cases, it is the ability to respond in real time to the state of the environment (i.e. phenotypic plasticity, which includes behaviour) that is expected to be transmitted rather than the response itself. This is, for example, the case with seasonal variation for an organism for which a generation takes several years.

Conversely, if a state of the environment persists for longer than one generation, then parents that are able to shape their offspring to be better adapted to that type of environment that will likely persist over their offspring's lifetime should have a selective advantage (**Figure 15**). For example, this is the case for seasonal variation for an organism with a generation time of a few hours, because at the timescale of this organism, seasonal variation is very slow and shows strong temporal autocorrelation with variation persisting over many generations. This is also the case for niche construction, where changes in the environment caused by the activities of organisms accumulate and persist beyond one generation.

Figure 15 also shows that two cases can be distinguished. On the one hand, non-genetic inheritance emerges clearly as suitable for the transmission of responses occurring at intermediate frequencies from changes occurring slightly less frequently than one generation, to those occurring relatively infrequently on the order of once every tens of thousands of generations (left part of **Figure 15**). And finally, genetic encoding appears to be the only process allowing the transmission of responses to changes that occur very rarely, on the order of less than once per ten thousand generations (right-hand side of **Figure 15**).

When theoretically exploring that question *Tobias Üller*, *Sinead Pen* and *Ido Pen* concluded that "*incomplete resetting* [of epigenetic marks] *between generations can evolve when the correlation of environmental states across generations* [i.e. the temporal autocorrelation of the environment] *is high and the accuracy of environmental cues is low*"[286]. This is because incomplete resetting protects against mismatched

phenotypes. What the recent discoveries on metabolic disorders such as diabetes have shown is that the resetting at the time of meiosis is probably thorough, but that some very specific epigenetic states are then acquired by the gametes through the acquisition of specific micro ARNs of somatic origin during their maturation. The concerned specific epigenetic states are directly involved in the accommodation with the specific environmental stress, implying that the environment can affect the information that is inherited. Nonetheless, that mechanism still leaves open the question of the incomplete resetting occurring later, such as at the time of fertilisation. This is an unresolved issue.

Environmental spatio-temporal autocorrelation, a missing piece of data

The question of the duration of a given environmental change is tantamount to asking the question of the spatial and temporal autocorrelation of the environment at the spatial and temporal scales of the organisms[287]. However, it is clear that there are relatively few studies of the spatial and temporal autocorrelation of the environment, and even fewer such studies at scales relevant to different organisms. There are many reasons for this, related to the complexity of such studies, but the fact remains that this is a major piece of ecological information that is sorely lacking.

Plasticity versus *heritability*

The above reasoning reveals a form of antinomy between plasticity and heritability in the sense that typically plasticity induces non-transmitted variation (which we will call V_{NT} in the next chapter) whereas inclusive heritability measures the transmitted part of the variation (which we will call V_T). This is why I avoid talking about transgenerational plasticity as some authors do to qualify situations such as those described in the second part of this book, as this can potentially generate a series of ambiguities.

Without conformity there is no science

One may be surprised by the sometimes very conformist behaviour of scientists, but in fact science would not exist without a certain amount of conformity (understood here as a resistance to new ideas). In fact, the absence of conformity would lead to the acceptance of any new idea, even the most far-fetched. There would then be no more information and no science. In fact, conformity participates to knowledge construction. It is up to the proponents of new ideas to sharpen their arguments to defend their vision, and then little by little the new ideas will be integrated into the mainstream vision. This transitional phase between an established science and an emerging science corresponds to what *Thomas Samuel Kuhn* called "extraordinary science", which marks the passage from one "normal science" to another[288].

A good example of the role of conformity in science is that of the study of the synapse, the structure that allows communication between neurons or between a neuron and a muscle cell. The accepted idea was that the synapse works electrically, rather like an electrical socket. However, researchers in the first half of the 20th century proposed that when a nerve impulse arrives at the synapse, it releases a molecule called a neurotransmitter into the tiny space between the upstream and downstream neurons, which, by affecting the functioning of the second neuron (or muscle), transmits the signal. According to this view, the synapse is not electrical but chemical. For 20 years, an Australian researcher named *John Eccles* defended the electrical synapse, forcing the proponents of the chemical synapse to make their case. However, in the meantime Eccles had met the philosopher of science *Karl Popper*, who explained that no scientific theory can be definitively held to be true because it has been verified by any number of experiments. According to Popper, a theory is only scientific if its statement can be disproved (or falsified) rather than verified as people usually say[289]. Impressed by this rigorous view, Eccles set out to invalidate his own theory of the electrical synapse, hoping of course not to succeed. But in 1951, at a meeting of the Philosophical Society in London, he was forced to admit that, against all odds, he had in fact invalidated his theory of the electrical synapse. He then became a fervent advocate of the chemical synapse. This reversal of position did not prevent him from winning the 1963 Nobel Prize in Physiology or Medicine, shared with *Alan Hodgkin* and *Andrew Huxley*. In a way, his 20 years of relentless opposition to the chemical synapse had advanced knowledge of the synapse to the point where he was awarded the Nobel Prize for his work on the synapse[290].

This constitutes a stimulating paradox. On the one hand, conformity can be seen as slowing science down; on the other hand, conformity is central to the scientific process by forcing researchers to try to falsify their hypotheses, for it is only when one succeeds in refuting (and not confirming) an idea that knowledge progresses.

Nature - nurture

Another comment that I have been facing is that the genetic (i.e. sequencic) non-genetic inheritance dichotomy is reminiscent of the old debate about nature (today usually understood as sequencic) *versus* nurture (here

usually understood as learned or acquired). Although I fully agree with that idea, I think three interconnected comments need to be made.

First, contrary to the nature-nurture debate, the more recent genetic-non-genetic inheritance dichotomy is deeply rooted in the detailed study of the molecular mechanisms of non-genetic inheritance. The recent debate clearly separates resemblance that results from the sharing of the same *sequencic* information by descent, from the one that results from the transmission of many other forms of non-sequencic information. This major clarification was made possible by the advent of high-throughput sequencing at the turn of the third millennium.

A second major difference between these two dichotomies concerns the position of the border between nature and nurture on the one hand and genetic and non-genetic on the other hand. A lot of case examples that clearly belonged to nature at the time of the nature-nurture debate today became part of non-genetic inheritance as all the examples of the second part of this book show. There is no doubt, for instance, that the inheritance of plastic responses that have been shown to be transmitted over many generations (such as the many example of *C. elegans* reported in **Box 3**) would have been considered as nature, while we now know that these are fascinating cases of non-genetic inheritance.

Finally, one may even question the relevance of drawing a border between two supposedly clearly distinct domains, as what emerges from **Figure 15** is that there is a continuum of timescales among all these processes. We can simplify that continuum with a dichotomy, and this is a bit what I do in **Figure 15**, but we should avoid opposing them, as these processes are complementary.

So my answer to that comment is, “correct, but now that we have far more detailed knowledge of the underlying mechanisms the frontiers between the two components of these dichotomies, if we are to draw them, actually lie in very different places along the gradient of transmission fidelity”. This, by the way, reveals the importance of studying the mechanisms that produce and transmit resemblance.

The Modern Synthesis of Evolution must be modernised

The many mechanisms detailed in the second part of this book seem to refute some aspects of the Modern Synthesis of Evolution in its present form[291]. Indeed, all these recent discoveries on non-genetic inheritance show that inheritance (or if you prefer transmitted resemblance) cannot be reduced to the mere transmission of the DNA sequence.

However, the fact remains that all the recent discoveries about non-genetic inheritance are, to say the least, surprising to both the general public and researchers and raise many questions that we listed at the end of **Chapter 8**. It is thus natural that these discoveries provoked a lot of backlash[292], as I report earlier in this chapter. For example, the person who, after my talk in March 2014 at the conference of the German Society for Human Genetics, attacked me head-on concluded his tirade by saying, "Please don't bring up the Lamarck debate again", even though I had been careful not to mention that name.

In such a situation of tension between an established and an emerging view, one could consider that those who deny the existence or importance of non-genetic inheritance are blinded by their certainties. This would have the advantage of blaming the existence of the conflict on only the other part of the community. However, one can wonder why the backlash is so violent. A significant part of this strong rejection of indeed surprising scientific findings is due to the way in which they are often presented. In the face of a mainstream that regularly rejects anything that seems to fall outside the scope of plausible phenomena within the dominant view, a natural reaction is to claim that the results challenge the dominant view, in this case the Modern Synthesis of Evolution, which is often tantamount to saying that it is intrinsically false. I must confess that I also adopted that attitude. However, the subliminal message behind such a formulation is that the reported results are of the utmost importance. It is certainly not up to the authors to assess the importance of their own findings. This is usually done by peers and historians, and we know how unfair history can be.

Furthermore, starting a discussion between alternative views by saying that the other view is wrong[293] is not the best way to get a new idea accepted. It would be much better to start by discussing all the commonalities and then address the question of how to fit the surprising results into the framework of the existing theory. This is what I try to do in this book.

In the present case, in order to be able to claim that non-genetic inheritance challenges the Modern Synthesis, one would need to have many more arguments than we currently have. In particular, it is necessary to illustrate how this is likely to change the very properties of evolution, and this can only be done through a series of complex approaches combining observation, experimentation and above all mathematical modelling.

In conclusion, if the existence of non-genetic inheritance challenges one of the foundations of the Modern Synthesis, according to which inheritance *in fine* can be reduced to the transmission of the DNA sequence, this does not mean that the Modern Synthesis is intrinsically false[294]. The Modern Synthesis was indeed a reductionist step, but a necessary one to advance our knowledge of inheritance and evolution. Most of the

knowledge acquired owing to this approach remains highly valuable and entirely valid. Even if the example of missing heritability has led us to question the purely sequencic interpretation of heritability, all the knowledge derived from population genetics, quantitative genetics and molecular genetics constitute indisputable pillars[295].

It is therefore not a matter of rejecting the current mainstream view of evolution but, on the contrary, of building on its undeniable acquisitions in order to imagine a new synthesis that would generalise the Modern Synthesis and make it compatible with all the other dimensions of inheritance currently known. The ambition of the emergence of a new synthesis, although too often presented as splitting, should be essentially unifying. The Modern Synthesis is the solid foundation on which to build a broader, more general and more integrative view of evolution. And if we are to make such an extension, we might as well do it inclusively so as to incorporate all known dimensions of transmitted resemblance. This is the ultimate goal of this third part. *We will do this in several steps, the first of which is shown in* ***Figure 15****,*

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Chapter 13
Sources of phenotypic variation

"*Any variation which is not inherited is unimportant for us*"
Darwin 1859 (page 12 of the original edition).

As far as I am concerned, all my ideas on inheritance emerged step by step following an observation that struck me as early as 1982, the year I joined the study of black-legged kittiwakes (*R. tridactyla*) breeding in the cliffs of *Cap Sizun*, the cape that runs from *Douarnenez* to *Audierne* via *Pointe du Raz* at the extreme west of the Eurasian continent. I had indeed noticed that breeder dispersal[296] behaviour was not only influenced by their personal experience but also, and very strongly so, by the experience of other breeders around them[297]. This result intrigued me because the literature on dispersal talked mainly about the role of personal experience, but did not mention the influence of the experience of neighbours. Such an observation was only possible thanks to the use of coloured rings, the combination of which allowed me to identify each individual, opening the way to a true individual monitoring as it is practised in humans.

I was fascinated immediately by the incredible morphological and behavioural variation within populations. For example, I could recognise individual kittiwakes by their voice. Similarly, a young male named 1WNBW (One-white-black-blue-white) in 1983 at the age of 3 years when males seek a female to breed, was so excited by the visits of females that his excitement caused them to flee. Similarly, another individual showed real signs of fear when landing on a breeding cliff, while others of the same age seemed at ease the first time I saw them landing among the nests of established breeders. In the same vein, during his thesis under my supervision at the end of the 1990s *Fabrice Helfenstein*, noticed that the black spots on the wingtips of the kittiwakes varied significantly among individuals. He thus developed a method to identify unringed birds, thus increasing the accuracy of the data he collected. In short, variation is everywhere in nature, and we have seen that it was the very existence of such ubiquitous variation that led Darwin and Wallace down the path of natural selection.

The aim of this chapter is to summarise the mechanisms responsible for this variation, focusing in particular on the part of the variation that is passed on to offspring. This is why I started this chapter by quoting Darwin's sentence, as I did in **Heredity concerns** patterns of parent-offspring resemblance. It is central to biology because natural selection and evolution cannot occur without heredity. It is thus vital to study the mechanisms that produce this resemblance that involves the transmission of many kinds of information from parents to offspring. Living organisms can therefore be defined as a 'memory machine' able to collect, store, use and then transmit a wide variety of environmental information. The study of heredity is therefore the study of the different forms of information that can be transmitted across generations and affect parent-offspring resemblance. However, during the 20th century, due to the fantastic discovery of the DNA molecule and its incredible sequencic memory properties, we became blind to the existence of other types of transmission mechanisms. As a result, we have increasingly reduced heredity to its sequencic component, i.e. the sole transfer of the information encoded into the nucleotidic sequence of DNA, an attitude that I call sequencic. It is now time to re-open our views of inheritance to approach it in all its complexity. A first step to achieve this goal is to reflect about the gene concept.

Chapter 2, because to my knowledge this is the first time that this question, which is so fundamental to the understanding of life, has ever been tackled.

How to decompose the variation among individuals

Darwin's sentence in fact distinguishes two components of variation, the part that is transmitted (which we will call V_T for transmitted variance) and the part that is not transmitted (V_{NT}, for non-transmitted variance). Darwin's sentence gives **Figure 16.A** with the addition that according to him non-transmitted variation can be ignored. This statement is a bit misleading, however, because part of non-transmitted variation reveals the accommodation to the environment (V_{NT} partly reveals phenotypic plasticity). But as Darwin wrote it in the context of natural selection, it is correct because, as although V_{NT} is about accommodation, it is not transmitted.

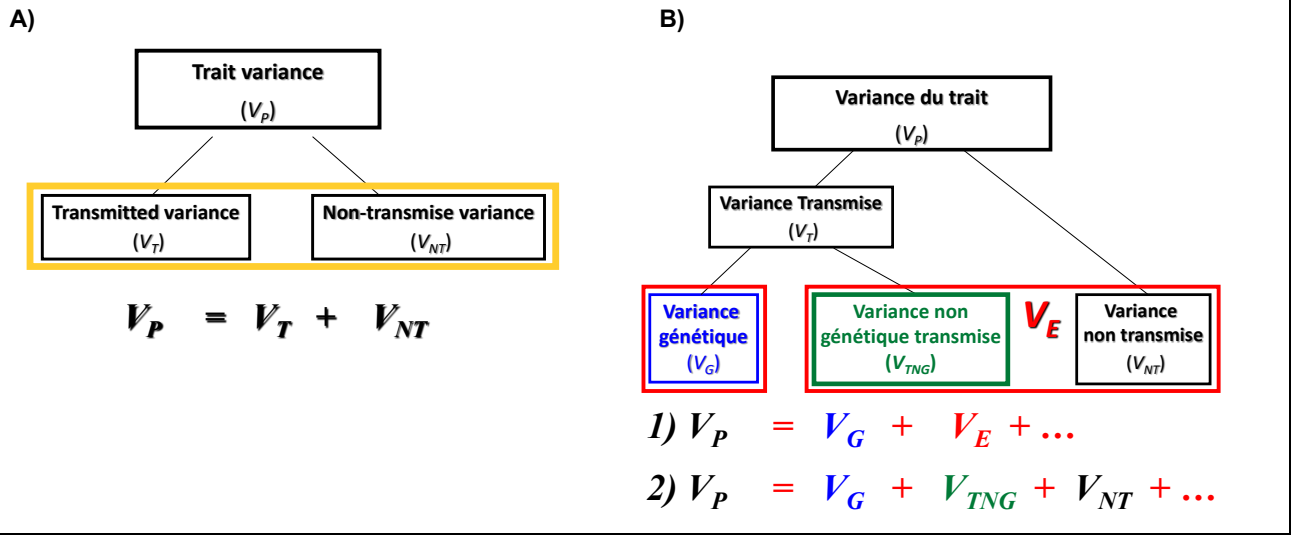
Since Darwin, we have made enormous progress in analysing the components of variation. In particular, we have added a very important box to this decomposition, that of genetic variation (the blue box labelled V_G in **Figure 16.B**) which today quantifies intra-population variation in the DNA sequence.

I would like to reiterate here that the discovery of genetics and its molecular avatar is probably one of the most fascinating discoveries of the 20th century in any science. However, as discussed earlier, this discovery has been so fascinating that it has gradually led us to neglect all other forms of inheritance and in particular to change the way we decompose phenotypic variance as revealed by the comparison of Figure 16, **A** and **B**.

A semantic shift from V_T to V_G

In practice, this has led us to ignore, and even often deny, that there may be other forms of inheritance, thus ignoring the existence of the green box in Figure 16,**B** (V_{TNG}) that depicts the part of phenotypic variation that is transmitted by non-sequencic mechanisms. Yet the V_{TNG} term in this decomposition encompasses the effect of all transmitted resemblance processes discussed in the second part of this book. I hope that at this point the reader is convinced of the reality and the fantastic complexity and subtlety as well as the real power of all these processes of inheritance which here are lumped into this green box.

Figure 16: Two decompositions of phenotypic variance. **A)** According to Darwin in yellow and **B)** according to the Modern Synthesis in red. The latter decomposition leads to equation 1, whereas the correct decomposition should be equation 2 that breaks down V_E into its transmitted (V_{TNG}) versus non-transmitted V_{NT} parts[298]. The three dots at the end of equations 1 and 2 visualise the fact that phenotypic variance can also be influenced by other effects, such as the interaction between genotype and environment (called G*E interaction) or by covariations between G and E. Here my aim is to keep it simple by focusing on the main effects.



The fascination with genetics has resulted in the fact that today, instead of decomposing phenotypic variance in such a way as to isolate the part V_T that is transmitted and can thus evolve over generations, we write the equation in a different way with the implicit goal of purifying the part of phenotypic variation that is due to sequencic variation (equation 1 in Figure 16,**B**). This formulation alone reveals the common belief that only the part that is genetically encoded can evolve over generations and therefore everything else is irrelevant. The comparison of these two decompositions reveals a profound and not necessarily conscious semantic shift that occurred during the second half of the 20th century, and in which, as we saw in the first part of this book, we are all more or less captive to today.

You said "Environmental variance"?

As a consequence, we have created the concept of environmental variance, which includes everything that is non-genetic and that we call V_E. This term, however, lumps components that play contrasting roles in accommodation, adaptation and evolution. One component of V_E is not transmitted (V_{NT}), the other (V_{TNG}) includes components that are not only transmitted, but in some cases are strongly transmitted, as is the case with the language we speak.

The concept of environmental variance is a source of great confusion. For example, for most biological researchers V_E correspond to V_{NT}, i.e. the non-transmitted part, which is incorrect. This was actually my case until the early 2000s, and I could not imagine that the processes summarised in the green box in Figure 16,**B** could exist. In fact, *it seems to me that this concept of environmental variance should be abandoned as it is too misleading to adopt the much clearer terminology of* Figure 16,**B**.

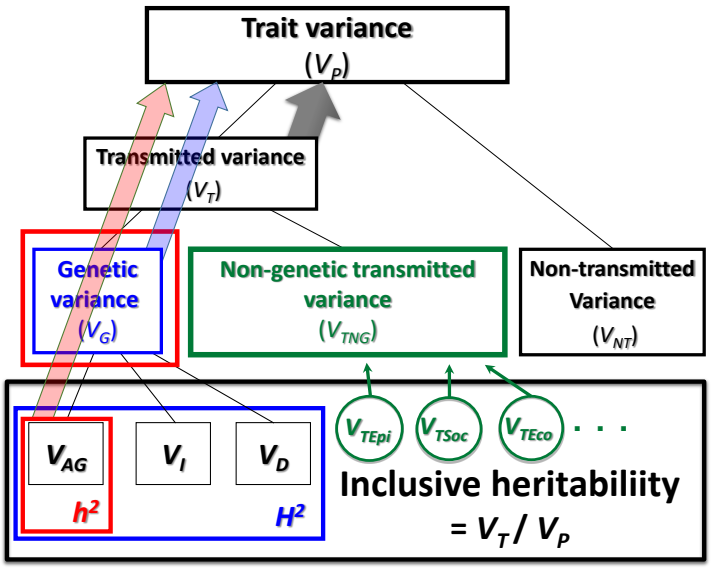
Discarding part of the baby with the bathwater

In fact, the implicit goal of decomposing phenotypic variance into V_G *versus* V_E is to get rid of V_E in order to estimate V_G. This is like discarding part of the baby with the bath water. Indeed, as an evolutionary biologist, the 'baby' we are interested in in this exercise is the variance that is transmitted (V_T), whatever the mechanism of transmission, whether genetic or non-genetic, because this is the part that can evolve.

Inclusive heritability

The variance decomposition exercise we have just carried out led us in 2010, with my colleague *Richard H. Wagner*, to propose a third concept of heritability which we called, after much hesitation, inclusive heritability (Figure 17).

Figure 17: The three concepts of heritability. Genetic variance has several components, including the additive genetic variance V_{AG}, which contributes to parent-offspring resemblance. The terms V_{TEpi}, V_{Tsoc}, and V_{TEco} correspond respectively to the variance generated by the transmitted part of epigenetic states, that resulting from social learning and that resulting from the transmission of the environment itself. I have only put these examples here, but as we shall see in Chapter 15, there are many other types of non-genetic mechanisms leading to transmitted resemblance. These terms are not meant to suggest that they should be estimated individually, as that would be impossible because they are far too intertwined. They merely point to areas of biology that provide evidence for the existence of V_{TNG}. Each of the three conceptions of heritability is visualised by an arrow connecting the two terms used to calculate each type of heritability. According to the classical view, narrow sense heritability (noted h^2) is measured by the ratio $h^2 = V_{AG} / V_P$ (thick pink arrows). However, in practice, it is very difficult, if not impossible, to access genetic additive variance. Therefore, broad sense heritability (denoted H^2) is most often used as being close to the ratio $H^2 \sim V_G / V_P$ (thick bluish arrow). We have proposed a third understanding of heritability, called inclusive heritability, which corresponds to the ratio of $IH^2 = V_T / V_P$ (thick grey arrow). Inclusive heritability therefore corresponds to the proportion of phenotypic variation that is transmitted, regardless of the transmission mechanism involved. This is the inheritance of differences all inclusive[299].



You will note that what we called inclusive heritability precisely corresponds to the original Darwinian concept. The aim is to capture the effects of all forms of transmitted resemblance. This is also the concept that implicitly interested our ancestors when they became farmers and sought to improve their domestic plants or animals.

Conclusion

Figure 16,**B** thus provides a framework and methodology that clarifies and generalises the classically practised decomposition of phenotypic variance by integrating as a separate term a component (V_G) that integrates the effect of sequencic variation, in parallel with a component (V_{TNG}) that is not related to variation in the DNA sequence but nonetheless transmitted.

*Figure 16,**B** constitutes a second step in the integration towards the Inclusive Evolutionary Synthesis.*

Chapter 14
life ⇔ memory.
Information at the heart of life

Since the beginning of this book we have discussed a number of circumstances where various types of information were found to persist across generations. In order of appearance, it was first sequencic information, then the information encoded in the stable 3D or even 4D structure of proteins and DNA. We have seen how parental behaviour and many environmental effects survive throughout life and beyond one generation. We have seen how social learning can make learned traits persist beyond one generation, giving rise to cultural inheritance. In doing so, we have talked about how intergenerational information flows build transmitted resemblance. Also, we have been interested in the nature and age of phenotypic variation. What do all these situations have in common? What could unify them in a single concept called information?

What unifies all these examples is not only the persistence of all these types of information, but also the persistence of the ability to retrieve this information and to use it appropriately. These are exactly the properties of 'memory', which sanctions the ability of an entity to store, retrieve and use information. Implicitly, in everyday life, we attribute this capacity to our brain, so that when a kid learns a poem we say that it uses and trains its memory. But in reality, isn't the notion of biological memory much broader than that? For example, should we not consider the encoding of information in the DNA sequence as a form of biological memory? Similarly, when the same DNA molecule is chemically modified, for example by adding methyl radicals to many cytosines, and these epigenetic marks are then passed on along cell lines or across generations, should we not also speak of biological memory? Furthermore, when the DNA sequence is transcribed into messenger RNA to produce a protein which, through its metabolic function, will affect the phenotype, it is as if the cell were recovering and mobilising its biological memory. In the same vein, when a protein takes on a stable 3D structure making it permanently non-functional, can we not consider this 3D structure as a form of biological memory? Finally, when we quantify inheritance by estimating inclusive heritability (i.e. transmitted resemblance), are we not measuring the intergenerational effects of various forms of biological memory? These are questions that we will address in this chapter.

Biological information

A first important observation is that the concepts of memory and of information are closely linked. Memory is about information persistence. Not only are these two concepts strongly linked, but they are often difficult to separate. All this book is about the mechanisms of inheritance which, as we have seen, involve the transmission of information across generations, thus producing transmitted resemblance, and paving the way for evolution by natural selection or drift. Reproduction thus appears as a process of intergenerational transfer of multiple types of information. Such inclusively heritable information thus exhibits remarkable intergenerational persistence, with some genes, for example, persisting unchanged for millions of generations.

But although the concept of information is absolutely central in biology, it is nonetheless very difficult to define. My purpose here is only to touch on it because it would justify a book in itself, which I would not be able to write. It would probably be impossible to find a consensus on what is meant by information in biology, because this concept can be described at too many structural scales. The concept of information therefore remains rather vague and multidimensional. This is also the case for the concept of fitness which, despite a definition that remains relatively vague or abstract, has enabled enormous developments in the study of evolution over the last 150 years. For instance, when *Richard Michod*, Professor of Evolutionary Ecology at the University of Arizona, came to Paris in the 1990s for a six-month sabbatical, he organised a dozen seminar-discussions on the sole concept of fitness. The diversity of views on this concept was truly amazing. In fact, both concepts, information and fitness, remain vague because their definition must be adapted to the biological question at hand, and there is probably no definition that is sufficiently generic to encompass all the subtleties of these concepts. Paradoxically, it is probably that characteristic that makes them so central.

Shannon's theory is not enough

In communication physics, there is the Shannon information theory, named after the person who established it in 1948[300]. Today there are other conceptions of information[301], but all of them have been developed in the context of human communication, among other things with the aim of optimising the transfer of information along a physical conductor such as telephone wires or through a more virtual conductor such as radio waves, telephone networks or Wi-Fi. Although these theories are not at all designed to capture the innumerable forms of biological information, many authors try to transpose it to biological information, which does not seem relevant to me.

For example, these theories are not easily transposed to communication among organisms. In behavioural ecology —the science about the evolution of behaviour— a distinction is often made between true communication, in which the signals used are the result of a history of selection, and communication in the broadest sense, which involves a range of social cues and which constitutes inadvertent social information extracted from the performance or state of other organisms[302]. Shannon's information theory may be able to describe true communication, but most likely is not able to describe inadvertent information, which nonetheless plays a major evolutionary role. For example, the mere presence of conspecifics in one habitat and not in another may provide valuable information that one habitat is favourable and the other probably less so.

Moreover, as we have seen in this book, the information involved in communication between individuals is only a very small part of the information involved in the development and functioning of biological entities. There are, however, interesting parallels. It is striking, for example, that hormones fit perfectly into the behaviourists' definition of signals; their current function of transmitting information within the organism emerged as a result of the evolutionary advantages of these circulating molecules in promoting the smooth internal functioning of organisms. They are therefore the result of a long history of selection and are therefore signals.

More generally, it is very difficult to define what unites these different forms of information (DNA sequence, non-coding RNA sequence, shape of macromolecules, hormones, nerve impulses, the finger pointing in a direction, a text engraved in a mud tablet etc.). Here, I will simply group them together under the term information.

A taxonomy of biological information

In 2010, we published with *Richard H. Wagner* an empirical essay on the taxonomy of biological information[303]. Given the complexity of the subject, our intention was to try to define some broad principles about biological information. It was one of the most difficult papers to complete in my career. I have since had the opportunity to return to it regularly, and I have found that what we wrote at the time stands the test of time, at least from my point of view.

Potential and realised information

In that essay we tried to define a general methodology for talking about biological information. For example, we drew a parallel with energy which can be potential or realised. Similarly, information can be potential or realised.

Any detectable fact or state of an entity in the environment can constitute potential information. But this information only becomes realised when interpreted by a living organism[304] that has detected this fact. Moreover, when a human observer claims that the organism under study has used this fact as *information about this or that*, another layer of interpretation of the situation is added, ours, which is far too subjective to be reliable. Two levels of subjectivity thus pile up, the organism's and ours. We therefore recommended that we always focus on potential information, i.e. on the initial facts or states used by an organism as a source of information, rather than on the interpretation and use made by the organism. In other words, we did not think it wise to talk about 'information for this or that', and recommended not to use this kind of formulation. Based on this principle, we proposed a classification and a definition, if possible generic, of the different types of biological information that we distinguished.

Information is a basic concept in biology

In 2005, with *Minus van Baalen* and *Jean Clobert*, both of whom were colleagues at the Functional and Evolutionary Ecology Laboratory of the Pierre and Marie Curie University in Paris, we believe that the concept of information is *The* primary concept in biology. Our idea is that it is perhaps the only concept necessary to approach the study of living organisms at all scales, from interactions between molecules to the functioning of ecosystems and the biosphere. In other words, it seems to us that it should be possible to rewrite all the major biological concepts —for example, gene expression, development, physiology, behaviour, ecology and evolution, fitness, inheritance— according to this single concept.

For example, heredity concerns transmitted resemblance and results from *the transfer of information across generations*. Inheritance is therefore the intergenerational transfer of information. Similarly, in population genetics we speak of gene flow among populations, but the real basic process that we seek to capture by this expression is not limited to the flow of sequencic information among populations, but rather refers to all types of information flow among populations, whether or not this information is sequencic in nature, heritable or not. This concerns, for example, epigenetic or cultural information flows, or microbial flows, or prion flows generated by the movement of individuals within a *metapopulation* (see Glossary). All such information flows are involved in ecological and evolutionary dynamics. Similarly, it seems to us that the concept of fitness could be reworked from an information perspective. It is for this reason that I drew a parallel between the concept of information and that of fitness earlier in this chapter, as these two concepts are closely linked.

I am just throwing out ideas here, which I agree are still rather vague, but our intuition with Minus and Jean is that this is a profound idea to be explored. I also think that this idea is integral to the Inclusive Evolutionary Synthesis to which the whole of this book, and more particularly this third part, is devoted.

The various forms of biological memory

Information is only meaningful if it is stored for a certain period of time. This time can be quite short, but it can also extend over an individual's entire life, or even over several or even millions of generations. For example, we saw in **Chapter 10**

Randomness and mutation

After discovering all these fascinating pathways of intergenerational information transfer, it is now necessary to develop an overlooked but basic property of epigenetic marks that is linked to a recurring issue in evolutionary biology, namely that of the randomness of mutations of all types. We have seen that one of the basic principles of the Modern Synthesis is that mutations are in no way directed by the environment towards improving the adaptation of organisms. Unfortunately, this principle is often simplified into saying that mutations occur at random, which does not mean the same thing. But what exactly is the case? This is what we will look at in this chapter.

Epigenetic marks are mutagenic...

The starting point that led me to think about the issue of mutation randomness was the fact that epigenetic marks, such as the presence of methyl radicals on cytosines, destabilises DNA and greatly increases the mutation rate of methyl-cytosines into thymine, another base of the DNA sequence. This, therefore, has the potential to generate point mutations whereby a cytosine is replaced by a thymine. Some articles have, for example, subheadings entitled "Methylation is mutagenic". For example, studies in humans suggest that cytosine methylation is responsible for 30-40% of point mutations in the human germline. Combining the results of several authors, cytosine methylation would increase the probability of cytosine mutating to thymine by a factor of about 20,000. This is such a considerable factor that it seems very unlikely that it is a negative collateral effect of a process selected in another context (in this case DNA methylation, which is involved in the regulation of gene expression). What then could be the function of a process that destabilises the fidelity of sequencic transmission to such an extent?

This is what we addressed in a 2019 paper. We proposed a mechanism by which such mutagenic power of DNA methylation, and more generally of epigenetic marks, might have provided a real evolutionary advantage by accelerating the sequencic engraving of the initially plastic responses to environmental conditions that prove to be very persistent. We have given this mechanism the explicit but unmemorable name of *epigenetically-facilitated mutational assimilation*.

Genetic assimilation

The idea of *genetic assimilation* (see Glossary) was proposed by *Conrad Waddington* following a series of experiments in *Drosophila* showing that following an environmental stress triggering an initially plastic response, this response tends to become heritable (and therefore non-plastic) after a certain number of generations under the effect of this stress. It was therefore as if, after a few dozen generations, characters initially developed in a plastic manner in response to a given environment became ‘genetically’ engraved, hence the expression 'genetic assimilation'.

Genetic or epigenetic assimilation?

However, it should be noted that in this expression the term genetic was understood in its pre-DNA sense, as 'that which is transmitted', without prejudging the mechanism responsible for this transmission. In particular, while Waddington's experiments undoubtedly demonstrated that the initially plastic trait became inclusively heritable, they did not at all show that this necessarily implied a sequencic change. In effect, there was nothing

in these experiments to suggest that what he observed at the phenotypic level resulted from a change in the DNA sequence. Given that Waddington had only worked over a few dozen generations —which was already a real challenge —he in fact most likely documented an "epigenetic assimilation" because the only thing his experiments really showed was that an initially plastic trait became inclusively inheritable within a few generations. This is equivalent to what *Mary Jane West-Eberhard* called "genetic accommodation" whereby a trait can be made heritable without necessarily involving encoding in the DNA sequence. Our paper proposed that, under certain conditions to which we will return later in this chapter, this process could go as far as sequencic engraving, *if the environmental stress persists over many, many generations*.

And the Modern Synthesis assimilated genetic assimilation

It has always puzzled me that the idea of genetic assimilation has finally been 'assimilated' by the Modern Synthesis, as this mechanism is strongly reminiscent of the much-rejected idea of inheritance of acquired traits. If you think about it, Waddington's mechanism proposes that within a few dozen generations under a given constant environmental stress the initially plastic response to stress can become heritable. In fact, what has allowed the idea of genetic assimilation to be assimilated is the relative slowness of this phenomenon. Moreover, the classical interpretation of this phenomenon is that there would pre-exist some neutral and hidden sequencic variation (usually called standing genetic variation) that would be somehow revealed by the environmental stress. Natural selection would then have the time to act over the few dozen generations of Waddington's experiments to retain only those variants that happen to be, I would like to say 'miraculously', favourable. So genetic assimilation would be just a special case of natural selection. This is how the Modern Synthesis has managed to see no major contradiction in genetic assimilation. This is also how I understood it until a few years ago.

Epigenetics as a hub towards sequencic engraving

A striking result on which we have built our reasoning is that all mechanisms of non-genetic heritability seem to involve some epigenetic change. It is as if epigenetics was the backbone or hub towards which most non-genetic inheritance processes would converge. Then, as epigenetic marks destabilize the DNA, over the course of many generations, this would generate sequencic variation *in the parts of the DNA concerned by the accommodation to the environmental change*. This would lead through natural selection acting on this newly produced variation, to sequencic engraving. In a way, epigenetics would be the conductor of the orchestra made up of all the genetic information. In effect, while it is very useful to have all the sequencic information (the recipe book), it is important to use it wisely. We shall see in **Chapter 16** that this epigenetic conductor is itself under the control of the brain.

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With *Arnaud Pocheville*, then based at the University of Sydney in Australia, we modelled this idea and were able to show that such a mechanism could accelerate the transfer of epigenetic encoding to sequencic encoding by a factor of the order of magnitude of the mutagenicity of the epigenetic marks, i.e. about 20,000 times. *This is what we called the epigenetically-facilitated mutational assimilation*.

But the story does not end there, as epigenetics interacts strongly with another major source of mutation, namely transposable elements.

... and interact with transposable elements

In parallel, we have been interested in another major phenomenon that can affect both the expression of certain genes and the appearance of mutations of all types. In fact, not only can the presence of epigenetic marks affect the stability of DNA, but epigenetic marks are themselves in strong interaction with the activity of transposable elements. Transposable elements are mobile DNA sequences discovered in maize by *Barbara McClintock* at the Cold Spring Harbor Laboratory on Long Island in the USA in the 1940s. This is one of the great genetic discoveries of the second half of the 20th century. There are a variety of transposable elements that differ, among other things, in the way they duplicate. Transposable elements exist in almost all living organisms. They seem to be able to invade the genome of an entire species through a process of colonisation from a local population, and can represent a large portion of the genome (about 15 to 22% in Drosophila, 40% of the genome in humans, and up to 90% in wheat). To give an idea of the prevalence of transposable elements, in humans, more than three million human sequences are derived from transposable elements, but only a few hundred of these have retained transposition capacity. The universality and mobility of transposable elements suggest that they play an important role in genome evolution and plasticity

The activity of transposable elements is under epigenetic control

The activity of transposable elements is strongly modulated by epigenetic processes (involving methylation, histone modifications or small RNAs) which are themselves affected by environmental factors. There are several hypotheses (not necessarily mutually exclusive) explaining the interaction between transposable

elements and epigenetics. In particular, the targeting of epigenetic modifications to transposable elements could be a consequence of the *exaptation* (see Glossary) of transposable elements as platforms for chromatin modification, in which case the epigenetic regulation of transposable elements could be a consequence of genome defence and regulation. As a result, environmental stresses can trigger transposition activity, either directly or through their effects on epigenetic marks associated with transposable elements. It can be said that in most cases the mobility of transposable elements is inhibited by epigenetic marks that block their replication. However, this targeting of epigenetic marks on transposable elements also affects, as if by ricochet, the genes close to these transposable elements —with which they become partners in a kind of "transposable-element-gene duo"—, thus affecting their expression level. Beyond their important mutational effects, by duplicating themselves in the genome, transposable elements can thus affect the general functioning of the genome, among other things by regulating and controlling the activity of genes in the neighbourhood of their insertion point. Thus transposable elements affect gene activity in three different ways.

- First, by attracting strong epigenetic marking around their insertion point, they affect the epigenetic marks, and therefore the expression, of the genes with which they are in duo. It should be noted that the epigenetic marks around transposable elements can be modified by stresses bringing back their mobility, hence modifying the expression of the genes around the new insertion point.
- On the other hand, as the sequence of many transposable elements carries regulatory elements of response to the environment, their presence will directly modulate the expression of the genes with which they are in duo according to the environmental context. They therefore play a central role in the response to environmental changes.
- Finally, by their mobility within the genome, transposable elements can generate significant sequencic changes in the genome. Their mutagenic potential is thought to increase the average point mutation rate by several tens of thousands of times.

A great generator of inclusively heritable variation

Thus, the presence of transposable elements in one area of the genome can on the one hand durably modify the expression of the surrounding genes due to the strong intervention of persistent epigenetic marks inhibiting their mobility, and on the other hand generate genetic (sequencic) variation in the whole genome as a result of their mobility. Both types of variation can affect the phenotype either negatively for individuals (e.g. they are implicated in various diseases) or positively at the population level by generating variation that is inclusively heritable and therefore open to selection. In other words, while at the individual level these changes can often have negative consequences, at the population level transposable elements generate inclusively heritable variation on which natural selection can act, thus favouring the adaptation of populations to their environment.

Interactions between epigenetics and transposable elements thus constitute a real engine for the creation of phenotypic variation (targeted to specific portions of the genome) that can be inherited either sequentially or epigenetically *in response to environmental stresses*, and are thus an important factor in evolution. Such a generator of genetic and epigenetic variation can in particular explain changes in mutability within the genome following environmental stresses. Several authors have emphasised the existence and importance of such generators of inclusively heritable variation involving the joint action of genetic and non-genetic processes in the ability of natural populations to adapt to ongoing global changes under the influence of human activities.

Epigenetically-facilitated mutational assimilation

We can now synthesize this. It appears that the effects of environmental stresses can affect the expression of specific genes involved in the response to stress and affect the activity of transposable elements, two major characteristics that each have the capacity to increase the sequencic mutation rate by tens of thousands of times, which is anything but negligible.

An information transfer pathway acting over many generations

The epigenetic changes affecting the expression of genes specifically involved in the response to an environmental stress in fact have two functions taking place on two very different time scale:

- First, these epigenetic marks, which we have seen target very precise portions of the DNA, enable the individual to adapt to the current environment by finely regulating the expression of the genes involved and leading to the phenotypic response to the environmental challenge. This response is rapidly established under the effect of environmental change. This process is known as phenotypic plasticity, the ability to modify the phenotype in response to the environment.
- Second, by being inherited, those epigenetic marks lastingly affect the mutability of the concerned genes that happen to be the genes involved in the accommodation to the specific environmental change. These epigenetic marks can also affect the activity of neighbouring transposable elements, which can further increase the mutability of the concerned regions and thus the potential generation of sequencic variation. In other words,

epigenetic marking would differentially mark portions of the genome for mutation, i.e. for the generation of sequencic variation and thus for the multigenerational exploration of new genetic possibilities. Far from being a cost in terms of evolution, this may on the contrary constitute a major evolutionary benefit because the sequencic variation thus generated concerns the genes actually involved in the accommodation to the specific environmental stress, a variation then open to natural selection.

This is *epigenetically-facilitated mutational assimilation* that is more than just a special case of natural selection on initially neutral and hidden genetic variation suddenly revealed by environmental change. According to our view, genetic assimilation appears as *a genuine mechanism for manufacturing sequencic variation in the parts of the genome concerned by the accommodation to the specific environment*, variation which is then open to natural selection. This mechanism calls for several important comments.

Random mutations in environmentally targeted areas of the genome

First, with epigenetically-facilitated mutational assimilation, the fundamental axiom of the Modern Synthesis that *mutations are not influenced by the environment in an adaptive direction* remains 100% valid. However, it is the simplified phrase traditionally used to simplify this axiom "mutations are random" that appears incorrect. With epigenetically-facilitated mutational assimilation the mutations generated following a lasting environmental change are indeed not influenced in an adaptive direction by the environment (the axiom of the Modern Synthesis therefore remains valid), but the parts of the genome where the mutation rate increases are actually targeted by the environment. *This is because epigenetic changes and the activity of transposable elements are themselves targeted by the environment*. There are therefore two independent scales where randomness can be expressed, that of regional portions of the DNA, and that of the local change of sequence itself. Only the second scale is unaffected by the environment, whereas the regional scale is clearly targeted by the effects of the environment in the sense that it is precisely in the portions of the DNA concerned by the accommodation to the environmental challenge that the mutation rate changes.

A necessarily slow process…

Second, even if the magnitude of several tens of thousands of increase in mutation rate seems enormous, it does not mean that epigenetically-facilitated mutational assimilation (i.e. the sequencic engraving of the adaptation) takes place in a few generations. A rough calculation predicts that such a process must take hundreds, if not thousands, of generations to become effective. Although the calculation proposed in the last note is very crude, the important point is that we should not expect epigenetically-facilitated mutational assimilation to take place very quickly, and certainly not in only a few tens of generations. And in fact, evolutionary logic even leads us to believe that this slowness is integral to the process (see below).

… which could be involved in domestication

We were certainly not the first to think about this type of genetic assimilation where the environment can be involved in generating genetic variation in the sections of the genome involved in the response to the environment. For example, one of the earliest papers on the subject dates back to 1983 in which *Hugh Iltis*, then Professor of Botany at the University of Wisconsin, formalised a scenario for the domestication of maize from teosinte, an annual plant from Central America. This remarkable scenario integrated several previous hypotheses and involved the major and massive effect of what he called a catastrophic epigenetic sexual transmutation that occurred some seven millennia ago.

Similarly, the whole literature on transposable elements claims that the environment can generate inclusively heritable variation. Regarding the idea that the environment can generate variation in certain regions of the genome, *Eva Jablonka* and her collaborators had modelled this idea without proposing a molecular mechanism. Similarly, *Michael Skinner* also foresaw and proposed the existence of such phenomena. Furthermore, researchers working on the domestication syndrome of vertebrates proposed that the stress induced at the beginning of domestication must have caused alterations in the methylation patterns of developmental genes expressed in the neural crest (the part of the embryo that will become the central nervous system), epigenetic changes that could have been fixed in the form of genetic variants to explain recurrent behavioural resemblances in the many domesticated fish, mammals and birds.

The different systems of inheritance interact with each other

This chapter thus introduced a particularly important point, namely that the different systems of inheritance (which we will summarise in **Chapter 15**) do not operate independently of each other. On the contrary, they interact and influence each other. For example, the central idea of epigenetically-facilitated mutational assimilation is that the molecular memory represented by epigenetics states interacts over the long term with sequencic memory, in a way that can potentially considerably accelerate the genetic encoding of initially plastic responses to environmental characteristics that persisted for hundreds or thousands of generations.

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Chapter 11, that the memorial dimension of social learning is central to the effective initiation of animal cultural, which itself can be seen as a memorial process on a population scale. This is the third criterion of our definition of animal culture.

Similarly, X-axis in **Figure 15** is a memory axis, showing that the study of inheritance is that of the various forms of long lasting memory. This is the case for all the forms of memory that we have seen in the second part of this book. The question of the different forms of biological memory is therefore central to any approach to biology in general and evolutionary biology in particular. Let us therefore take the time to recapitulate without any ambition of exhaustiveness.

A memory machine

As we have seen, by memory we mean the capacity of an entity to retain and retrieve information. By extension, the capacity of living organisms to transmit and use information across generations can therefore be described as intergenerational memory. This information is encoded in stable states of molecules or structures such as cells, tissues or even organs. It is these stable states that provide the memory function. We can then look at life as a formidable memory machine acting at different timescales ranging from a fraction of a second to millions of generations. Hence the title of this chapter, which can be read in both directions: life implies the existence of memory and memory makes life.

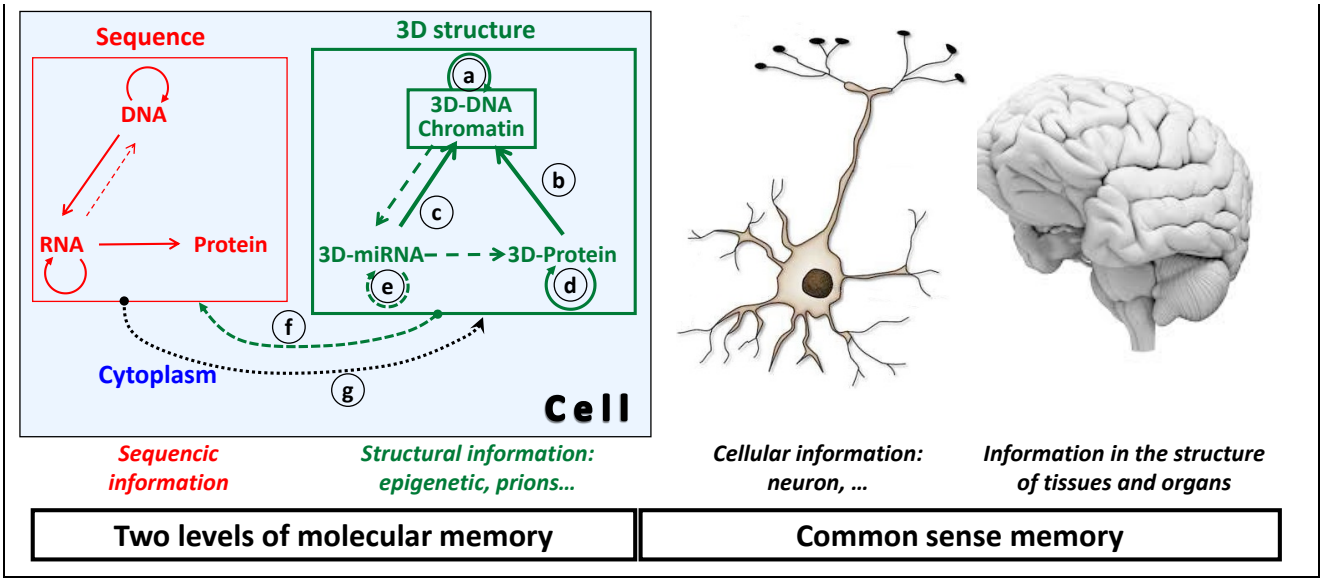
Molecular memories

Four main levels of biological memory can be distinguished broadly: the memory engraved in the sequence of organic molecules (proteins and macromolecules, in red in **Figure 18**), the memory based on stable configurations of these same macromolecules (in green in **Figure 18**), the memory based on stable configurations of living cells, and the memory based on the stable configuration of tissues within organs (**Figure 18**).

The central dogma of molecular biology: sequencic memories

In 1958 and again in 1970 *Francis Crick*, one of the four discoverers of the structure of the DNA molecule, proposed what is known as the central dogma of molecular biology[305]. This is easily represented in the form of a diagram, shown in red in **Figure 18**, which sets out the direction of sequencic information transfers between the three major groups of fundamental molecules of life, proteins, RNA and DNA. Only those transfers represented by arrows are possible. For example, we still have no argument that a protein from another organism can, by its mere presence in a recipient organism, allow it to be encoded in the form of a new DNA sequence integrated into the genome of the recipient organism, which would then lead to the lasting memorization in the recipient organism of this new protein and which would constitute an ultimate form of parasitism. There is therefore no arrow from protein to DNA in this diagram that represents the lowest structural level of storage of biological information. It concerns only the primary (i.e. sequencic) structure of molecules, either of nucleotides or of amino acids, and is the subject of numerous studies. This level involves a digitised memory in the form of a sequencic code which differs according to the type of macromolecule, a base 4 system for DNA and RNA, and a base 20 plus (the different types of amino acids) for proteins.

Figure 18: Main types of biological memory. Information may persist for only a short time, or for a lifetime, or even well beyond one generation. This figure is by no means exhaustive. It illustrates 1) that memory is based on a large number of molecular, cellular and anatomical mechanisms, and 2) that information flows at a given structural level may be impossible at other levels and vice versa. The red part takes up ‘with only one small modification) the diagram of the central dogma of molecular biology enunciated by *Francis Crick*, which concerns sequencic information exclusively. The green part uses the same formalism for information flows at the level of the 3D structure of molecules 2019[306]. See the text for the rationale of the various arrows in the green part. At a higher structural scale, we know that learning and long-term memory involve protein syntheses, which reinforces specific synapses possibly at the expense of others (hence the image of a neuron). Finally, we know that the general structure of the brain as a set of different tissues plays a major role in common sense memory. It is, for example, this last form of memory that seems to be most involved in culture. All these forms of memory may be involved in inheritance.



The memories engraved in the 3D or even 4D structure of the fundamental organic molecules

The second structural level of encoding and memorising information concerns the tertiary structure of these same three types of organic molecules (in green in **Figure 18**). At this level, the information is more analogous in nature because at this level the epigenetic marks are very numerous and can be combined in an infinite number of different states. Comparing the red and green parts of this diagram, it is immediately striking that the possible paths for 3D information transfer (green) are very different from those at the sequencic level (red).

Arrow (a) illustrates that epigenetic marks are duplicated during DNA duplication, together with sequence duplication. This is particularly the case during mitosis, and it is this memory that allows cell differentiation and organ formation in multi-cellular organisms. Arrow (b) represents the major role of proteins in the 3D structure of chromatin. This is, for example, the case of histones associated in groups of eight to form a nucleosome around which the long DNA chain is wound over 146 base pairs (see **However,** before going into the description of these many striking examples, it is necessary to take the time to introduce a fascinating and rapidly growing field of organismal biology, that of epigenetics.

). Overall, chromatin involves a wide range of proteins that affect its 3D configuration. Arrow (c) represents the ability of non-coding RNAs to strongly affect the chromatin structure of very specific parts of the genome. Arrow (d) represents the ability of some proteins to transfer their configuration to other proteins (as in prions) or to stabilise other proteins in a functional configuration (chaperone molecule). Arrow (e) represents the fact that some non-coding RNAs can self-replicate[307]. Arrow (f) represents the effect of epigenetically-facilitated mutational assimilation, which we described in **Chapter 10**[308]. This arrow therefore represents the shift from rather shorter-term memory (over hundreds of generation) to very long-term memory (over millions of generations). Arrow (g) represents the fact that the sequence structure of molecules affects the 3D structure and thus the biological function of the three main categories of organic molecules. Evidence for all these arrows was provided in the second part of this book.

Memories recorded in cells and their connections

With this new type of memory we come very close to the common meaning given to the concept of memory for organisms with neurons possibly structured to form a central nervous system. It is in fact the memory embedded in the structure and configuration of the cells themselves. Here, I have represented the case of a neuron because it is the typical example, but the same type of memory potentially exists in any type of cell. There is a whole literature on the question of cellular memory, both in unicellular and multicellular organisms (e.g. lymphocyte memory in immunity)[309]. Cellular, epigenetic and genetic memories are the main types of memory in single-celled organisms.

In multi-cellular organisms, memory can be stored in the layout of cells within tissues, and in organisms with a brain, there is also common sense memory, which emerges from the structure and functioning of the central nervous system based on learning involving the functioning of the brain itself. Connections between neurons (i.e. synapses) play a major role in this type of memory. It is the type of memory that I am soliciting in the reader with this book. All these forms of memory can participate in transmitted resemblance and therefore in inheritance and evolution by natural selection or drift.

All memory types are stored in the configuration of biological entities

Interestingly, memory can also emerge at the larger scale of the group in the form of cultural memory. A striking point is that in all cases, the avatar of information is the configuration, i.e. in the form taken by a biological entity. Configuration can encode information digitally, as in the case of sequence information, or analogically, as in the case of the 3D structure of macromolecules or cellular memory or that embodied in the structure of tissues and organs. Similarly, cultural memory is engraved in the group structuration and in the nature of the interactions among group members. However, in all cases, these states or configurations are part of memory because they are more or less stable over time, which means that energy must be spent to bring them out of these particular states (a bit like if these states were at the bottom of a more or less deep well). Moreover, in a certain number of cases, this configuration is transmitted either by contact (as in the case of prions), or because when one of these entities produces a daughter entity, the latter inherits the same state or configuration as the parent entity.

Information and memory are at the heart of life

The Modern Synthesis is only interested in the first form of biological memory, the one whose avatar is the DNA sequence. In fact, we must integrate all the other forms of biological memory (epigenetic, i.e. the 3D or even 4D structure of macromolecules, prions, cellular, tissue and organ memories, as well as ecological and cultural memory) which are mostly assumed to be either non-existent or negligible on an intergenerational scale. This is an assumption that is proving to be wrong, and once an error is identified, we should correct it. In other words, we need to make further progress in integrating into our reasoning all forms of biological memory participating in transmitted resemblance, setting the stage for evolution. Thus, **Figure 18**, *provides a third dimension that must be integrated into the Inclusive Synthesis of Evolution.*

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Chapter 15
The multiple pathways of inheritance

In the second part of this book, to account for all the cases of non-genetic transmission we have added several pathways to the diagram of transgenerational information flows as classically understood within the Modern Synthesis of Evolution (presented in Erreur ! Source du renvoi introuvable.**B**). What would this diagram look like if we put all the different pathways of inheritance into a single integrative diagram representing as much as possible all the documented information flows across generations that may participate in transmitted resemblance?

For this purpose, our starting point is therefore the diagram in Erreur ! Source du renvoi introuvable.**B** in which there was only one red arrow of intergenerational transmission, that by which the information encoded in the DNA sequence is transmitted to offspring by gametes. The synthesis of all documented types of flow is shown in Figure 19, that differs strikingly from that of Erreur ! Source du renvoi introuvable.**B**. In this chapter, using arrow numbers, we will recapitulate all these arrows, in particular arrows not yet described.

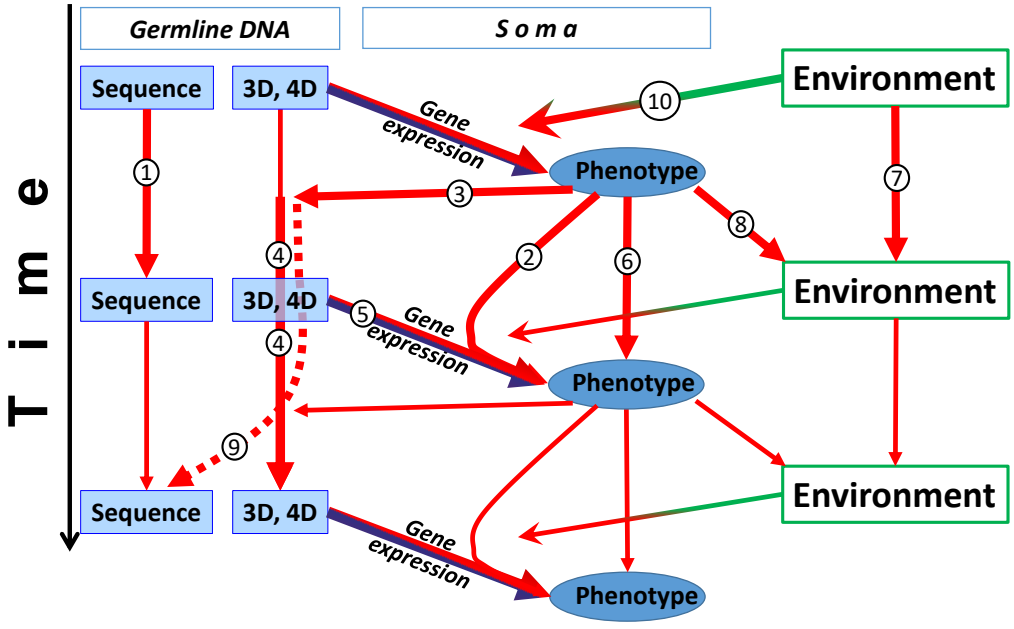
Genetic transmission (arrow 1)

Arrow 1 shows the path taken by sequencic information. There is no need to dwell on it as it is undoubtedly the best known as it has been the focus of the study of inheritance and evolution for over the last 100 years.

Reconstructing the same trait under the direct parental effects (arrow 2)

We already introduced arrow 2 in Erreur ! Source du renvoi introuvable., where we saw how mothers, by caring or not caring for their young, constitute an element of their offspring environment that induces specific epigenetic chance in the brains of their daughters, which then lead the latter to reconstruct the same trait as their mother (in this case, the behaviour of caring for their young) once adults. We have seen that such a form of inheritance concerns many behavioural patterns and does not involve information conveyed by gametes.

Figure 19: **Diagram of information flows across generations according to the Inclusive Evolutionary Synthesis**. This diagram represents three generations and two transmission steps. Comparison with Erreur ! Source du renvoi introuvable.**B**, which shows the same diagram according to the Modern Synthesis, shows the multiplicity of the documented pathways of information transfer across generations, each making its own contribution to inheritance. The numbers on the red arrows correspond to the numbers used in the section headings in this chapter. The numbered arrows are thicker for better visualization, but are of course exactly the same as in the next generation. The explanation for the double colour of arrows 5 and 10 is given in the text[310].



The inheritance of numerous plastic responses (arrows 3, 4 and 5)

We saw in **Chapter 7** to **Chapter 9** when presenting the inheritance of several metabolic disorders such as type II diabetes the reason for the introduction of arrows 3, 4 and 5 in **Figure 19**. This inheritance pathway involves three elements acting in series.

- The first step (arrow 3) is that an environmental change/stress can lead to the making and diffusion within the body of small non-coding RNAs(see **Box 2**) able to alter the epigenetic state of certain genes involved in the response to that environmental change/stress, either in somatic cells or in gametes. Arrow 3 constitutes a true soma-to-germline communication pathway [i.e. between the non-germ part of an organism (soma) and its germ part (germline)]. In the next chapter, we will discuss the origin of such small non-coding RNAs.
- The second step (arrow 4) is that some of these acquired epigenetic states in the germline that can persist for several generations and even up to at least a hundred generations (as has been sometimes shown). Arrow 4 visualises such epigenetic inheritance. It begins at the point where arrow 3 ends and continues through the following generations.
- The third step is that the epigenetic changes induced in the gametes subsequently affect the development of the offspring and thus induce changes in the offspring phenotype (red part of arrow 5, **Figure 19**). Arrow 5 therefore has two colours to reveal its dual functions, black as it visualises development, and red for its involvement in inheritance as we saw in **Chapter 7** to **Chapter 9**. The importance of arrow 5 is beautifully illustrated and detailed in Wallace Arthur's excellent book[311].

Cultural inheritance and its great originality (arrow 6)

We saw in Chapter 10

Randomness and mutation

After discovering all these fascinating pathways of intergenerational information transfer, it is now necessary to develop an overlooked but basic property of epigenetic marks that is linked to a recurring issue in evolutionary biology, namely that of the randomness of mutations of all types. We have seen that one of the basic principles of the Modern Synthesis is that mutations are in no way directed by the environment towards improving the adaptation of organisms. Unfortunately, this principle is often simplified into saying that mutations occur at random, which does not mean the same thing. But what exactly is the case? This is what we will look at in this chapter.

Epigenetic marks are mutagenic...

The starting point that led me to think about the issue of mutation randomness was the fact that epigenetic marks, such as the presence of methyl radicals on cytosines, destabilises DNA and greatly increases the mutation rate of methyl-cytosines into thymine, another base of the DNA sequence. This, therefore, has the potential to generate point mutations whereby a cytosine is replaced by a thymine. Some articles have, for example, subheadings entitled "Methylation is mutagenic". For example, studies in humans suggest that cytosine methylation is responsible for 30-40% of point mutations in the human germline. Combining the results of several authors, cytosine methylation would increase the probability of cytosine mutating to thymine by a factor of about 20,000. This is such a considerable factor that it seems very unlikely that it is a negative collateral effect of a process selected in another context (in this case DNA methylation, which is involved in the regulation of gene expression). What then could be the function of a process that destabilises the fidelity of sequencic transmission to such an extent?

This is what we addressed in a 2019 paper. We proposed a mechanism by which such mutagenic power of DNA methylation, and more generally of epigenetic marks, might have provided a real evolutionary advantage by accelerating the sequencic engraving of the initially plastic responses to environmental conditions that prove to be very persistent. We have given this mechanism the explicit but unmemorable name of *epigenetically-facilitated mutational assimilation*.

Genetic assimilation

The idea of *genetic assimilation* (see Glossary) was proposed by *Conrad Waddington* following a series of experiments in *Drosophila* showing that following an environmental stress triggering an initially plastic response, this response tends to become heritable (and therefore non-plastic) after a certain number of generations under the effect of this stress. It was therefore as if, after a few dozen generations, characters initially developed in a plastic manner in response to a given environment became 'genetically' engraved, hence the expression 'genetic assimilation'.

Genetic or epigenetic assimilation?

However, it should be noted that in this expression the term genetic was understood in its pre-DNA sense, as 'that which is transmitted', without prejudging the mechanism responsible for this transmission. In particular, while Waddington's experiments undoubtedly demonstrated that the initially plastic trait became inclusively heritable, they did not at all show that this necessarily implied a sequencic change. In effect, there was nothing in these experiments to suggest that what he observed at the phenotypic level resulted from a change in the DNA sequence. Given that Waddington had only worked over a few dozen generations —which was already a real challenge —he in fact most likely documented an "epigenetic assimilation" because the only thing his experiments really showed was that an initially plastic trait became inclusively inheritable within a few generations. This is equivalent to what *Mary Jane West-Eberhard* called "genetic accommodation" whereby a trait can be made heritable without necessarily involving encoding in the DNA sequence. Our paper proposed that, under certain conditions to which we will return later in this chapter, this process could go as far as sequencic engraving, *if the environmental stress persists over many, many generations*.

And the Modern Synthesis assimilated genetic assimilation

It has always puzzled me that the idea of genetic assimilation has finally been 'assimilated' by the Modern Synthesis, as this mechanism is strongly reminiscent of the much-rejected idea of inheritance of acquired traits. If you think about it, Waddington's mechanism proposes that within a few dozen generations under a given constant environmental stress the initially plastic response to stress can become heritable. In fact, what has allowed the idea of genetic assimilation to be assimilated is the relative slowness of this phenomenon. Moreover, the classical interpretation of this phenomenon is that there would pre-exist some neutral and hidden sequencic variation (usually called standing genetic variation) that would be somehow revealed by the environmental stress. Natural selection would then have the time to act over the few dozen generations of Waddington's experiments to retain only those variants that happen to be, I would like to say 'miraculously', favourable. So genetic assimilation would be just a special case of natural selection. This is how the Modern Synthesis has managed to see no major contradiction in genetic assimilation. This is also how I understood it until a few years ago.

Epigenetics as a hub towards sequencic engraving

A striking result on which we have built our reasoning is that all mechanisms of non-genetic heritability seem to involve some epigenetic change. It is as if epigenetics was the backbone or hub towards which most non-genetic inheritance processes would converge. Then, as epigenetic marks destabilize the DNA, over the course of many generations, this would generate sequencic variation *in the parts of the DNA concerned by the accommodation to the environmental change*. This would lead through natural selection acting on this newly produced variation, to sequencic engraving. In a way, epigenetics would be the conductor of the orchestra made up of all the genetic information. In effect, while it is very useful to have all the sequencic information (the recipe book), it is important to use it wisely. We shall see in **Chapter 16** that this epigenetic conductor is itself under the control of the brain.

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With *Arnaud Pocheville*, then based at the University of Sydney in Australia, we modelled this idea and were able to show that such a mechanism could accelerate the transfer of epigenetic encoding to sequencic encoding by a factor of the order of magnitude of the mutagenicity of the epigenetic marks, i.e. about 20,000 times. *This is what we called the epigenetically-facilitated mutational assimilation*.

But the story does not end there, as epigenetics interacts strongly with another major source of mutation, namely transposable elements.

... and interact with transposable elements

In parallel, we have been interested in another major phenomenon that can affect both the expression of certain genes and the appearance of mutations of all types. In fact, not only can the presence of epigenetic marks affect the stability of DNA, but epigenetic marks are themselves in strong interaction with the activity of transposable elements. Transposable elements are mobile DNA sequences discovered in maize by *Barbara McClintock* at the Cold Spring Harbor Laboratory on Long Island in the USA in the 1940s. This is one of the great genetic discoveries of the second half of the 20th century. There are a variety of transposable elements that differ, among other things, in the way they duplicate. Transposable elements exist in almost all living organisms. They seem to be able to invade the genome of an entire species through a process of colonisation from a local population, and can represent a large portion of the genome (about 15 to 22% in Drosophila, 40% of the genome in humans, and up to 90% in wheat). To give an idea of the prevalence of transposable elements, in humans, more than three million human sequences are derived from transposable elements, but only a few hundred of these have retained transposition capacity. The universality and mobility of transposable elements suggest that they play an important role in genome evolution and plasticity

The activity of transposable elements is under epigenetic control

The activity of transposable elements is strongly modulated by epigenetic processes (involving methylation, histone modifications or small RNAs) which are themselves affected by environmental factors. There are several hypotheses (not necessarily mutually exclusive) explaining the interaction between transposable elements and epigenetics. In particular, the targeting of epigenetic modifications to transposable elements could be a consequence of the *exaptation* (see Glossary) of transposable elements as platforms for chromatin modification, in which case the epigenetic regulation of transposable elements could be a consequence of genome defence and regulation. As a result, environmental stresses can trigger transposition activity, either directly or through their effects on epigenetic marks associated with transposable elements. It can be said that in most cases the mobility of transposable elements is inhibited by epigenetic marks that block their replication. However, this targeting of epigenetic marks on transposable elements also affects, as if by ricochet, the genes close to these transposable elements —with which they become partners in a kind of "transposable-element-gene duo"—, thus affecting their expression level. Beyond their important mutational effects, by duplicating themselves in the genome, transposable elements can thus affect the general functioning of the genome, among other things by regulating and controlling the activity of genes in the neighbourhood of their insertion point. Thus transposable elements affect gene activity in three different ways.

- First, by attracting strong epigenetic marking around their insertion point, they affect the epigenetic marks, and therefore the expression, of the genes with which they are in duo. It should be noted that the epigenetic marks around transposable elements can be modified by stresses bringing back their mobility, hence modifying the expression of the genes around the new insertion point.
- On the other hand, as the sequence of many transposable elements carries regulatory elements of response to the environment, their presence will directly modulate the expression of the genes with which they are in duo according to the environmental context. They therefore play a central role in the response to environmental changes.
- Finally, by their mobility within the genome, transposable elements can generate significant sequencic changes in the genome. Their mutagenic potential is thought to increase the average point mutation rate by several tens of thousands of times.

A great generator of inclusively heritable variation

Thus, the presence of transposable elements in one area of the genome can on the one hand durably modify the expression of the surrounding genes due to the strong intervention of persistent epigenetic marks inhibiting their mobility, and on the other hand generate genetic (sequencic) variation in the whole genome as a result of their mobility. Both types of variation can affect the phenotype either negatively for individuals (e.g. they are implicated in various diseases) or positively at the population level by generating variation that is inclusively heritable and therefore open to selection. In other words, while at the individual level these changes can often have negative consequences, at the population level transposable elements generate inclusively heritable variation on which natural selection can act, thus favouring the adaptation of populations to their environment.

Interactions between epigenetics and transposable elements thus constitute a real engine for the creation of phenotypic variation (targeted to specific portions of the genome) that can be inherited either sequentially or epigenetically *in response to environmental stresses*, and are thus an important factor in evolution. Such a generator of genetic and epigenetic variation can in particular explain changes in mutability within the genome following environmental stresses. Several authors have emphasised the existence and importance of such generators of inclusively heritable variation involving the joint action of genetic and non-genetic processes in the ability of natural populations to adapt to ongoing global changes under the influence of human activities.

Epigenetically-facilitated mutational assimilation

We can now synthesize this. It appears that the effects of environmental stresses can affect the expression of specific genes involved in the response to stress and affect the activity of transposable elements, two major characteristics that each have the capacity to increase the sequencic mutation rate by tens of thousands of times, which is anything but negligible.

An information transfer pathway acting over many generations

The epigenetic changes affecting the expression of genes specifically involved in the response to an environmental stress in fact have two functions taking place on two very different time scale:

- First, these epigenetic marks, which we have seen target very precise portions of the DNA, enable the individual to adapt to the current environment by finely regulating the expression of the genes involved and leading to the phenotypic response to the environmental challenge. This response is rapidly established under

the effect of environmental change. This process is known as phenotypic plasticity, the ability to modify the phenotype in response to the environment.

- Second, by being inherited, those epigenetic marks lastingly affect the mutability of the concerned genes that happen to be the genes involved in the accommodation to the specific environmental change. These epigenetic marks can also affect the activity of neighbouring transposable elements, which can further increase the mutability of the concerned regions and thus the potential generation of sequencic variation. In other words, epigenetic marking would differentially mark portions of the genome for mutation, i.e. for the generation of sequencic variation and thus for the multigenerational exploration of new genetic possibilities. Far from being a cost in terms of evolution, this may on the contrary constitute a major evolutionary benefit because the sequencic variation thus generated concerns the genes actually involved in the accommodation to the specific environmental stress, a variation then open to natural selection.

This is *epigenetically-facilitated mutational assimilation* that is more than just a special case of natural selection on initially neutral and hidden genetic variation suddenly revealed by environmental change. According to our view, genetic assimilation appears as *a genuine mechanism for manufacturing sequencic variation in the parts of the genome concerned by the accommodation to the specific environment*, variation which is then open to natural selection. This mechanism calls for several important comments.

Random mutations in environmentally targeted areas of the genome

First, with epigenetically-facilitated mutational assimilation, the fundamental axiom of the Modern Synthesis that *mutations are not influenced by the environment in an adaptive direction* remains 100% valid. However, it is the simplified phrase traditionally used to simplify this axiom "mutations are random" that appears incorrect. With epigenetically-facilitated mutational assimilation the mutations generated following a lasting environmental change are indeed not influenced in an adaptive direction by the environment (the axiom of the Modern Synthesis therefore remains valid), but the parts of the genome where the mutation rate increases are actually targeted by the environment. *This is because epigenetic changes and the activity of transposable elements are themselves targeted by the environment*. There are therefore two independent scales where randomness can be expressed, that of regional portions of the DNA, and that of the local change of sequence itself. Only the second scale is unaffected by the environment, whereas the regional scale is clearly targeted by the effects of the environment in the sense that it is precisely in the portions of the DNA concerned by the accommodation to the environmental challenge that the mutation rate changes.

A necessarily slow process…

Second, even if the magnitude of several tens of thousands of increase in mutation rate seems enormous, it does not mean that epigenetically-facilitated mutational assimilation (i.e. the sequencic engraving of the adaptation) takes place in a few generations. A rough calculation predicts that such a process must take hundreds, if not thousands, of generations to become effective. Although the calculation proposed in the last note is very crude, the important point is that we should not expect epigenetically-facilitated mutational assimilation to take place very quickly, and certainly not in only a few tens of generations. And in fact, evolutionary logic even leads us to believe that this slowness is integral to the process (see below).

… which could be involved in domestication

We were certainly not the first to think about this type of genetic assimilation where the environment can be involved in generating genetic variation in the sections of the genome involved in the response to the environment. For example, one of the earliest papers on the subject dates back to 1983 in which *Hugh Iltis*, then Professor of Botany at the University of Wisconsin, formalised a scenario for the domestication of maize from teosinte, an annual plant from Central America. This remarkable scenario integrated several previous hypotheses and involved the major and massive effect of what he called a catastrophic epigenetic sexual transmutation that occurred some seven millennia ago.

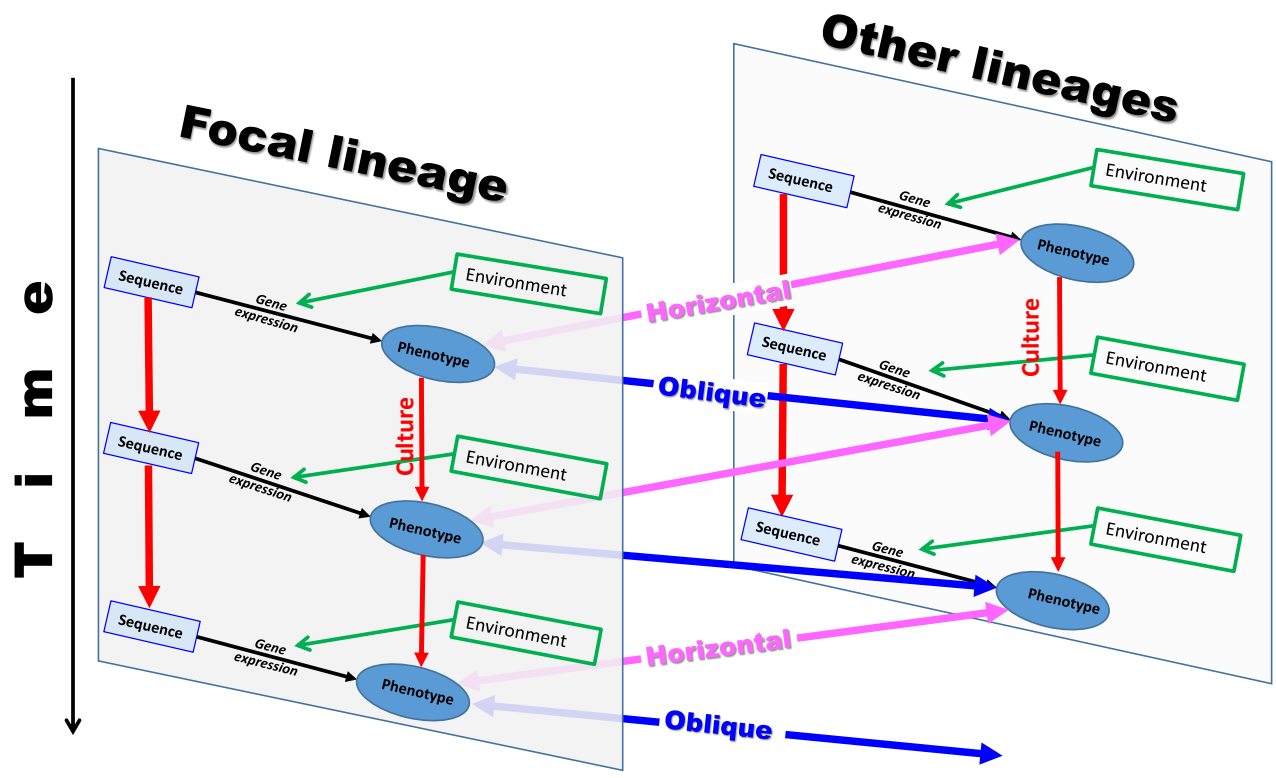
Similarly, the whole literature on transposable elements claims that the environment can generate inclusively heritable variation. Regarding the idea that the environment can generate variation in certain regions of the genome, *Eva Jablonka* and her collaborators had modelled this idea without proposing a molecular mechanism. Similarly, *Michael Skinner* also foresaw and proposed the existence of such phenomena. Furthermore, researchers working on the domestication syndrome of vertebrates proposed that the stress induced at the beginning of domestication must have caused alterations in the methylation patterns of developmental genes expressed in the neural crest (the part of the embryo that will become the central nervous system), epigenetic changes that could have been fixed in the form of genetic variants to explain recurrent behavioural resemblances in the many domesticated fish, mammals and birds.

The different systems of inheritance interact with each other

This chapter thus introduced a particularly important point, namely that the different systems of inheritance (which we will summarise in **Chapter 15**) do not operate independently of each other. On the contrary, they interact and influence each other. For example, the central idea of epigenetically-facilitated mutational assimilation is that the molecular memory represented by epigenetics states interacts over the long term with sequencic memory, in a way that can potentially considerably accelerate the genetic encoding of initially plastic responses to environmental characteristics that persisted for hundreds or thousands of generations.

Chapter 11 that cultural inheritance could be visualised in this diagram by arrow 6. But we also saw that this formalism could suggest that the properties of cultural transmission are very similar to those of genetic transmission.

Figure 20: Cultural transmission is the only type of inheritance that allows frequent transfers of information across family lines. 'Horizontal' refers to transmission across individuals of the same generation but belonging to different family lines. 'Oblique' refers to transmission among unrelated individuals of different generations. For the sake of readability, the flow diagrams include only the genetic and cultural components (as in Figure 12), but a complete diagram should include all transmission routes from Figure 19.



In fact, the flow chart in Figure 19 represents what happens along a single family line over three generations. To visualise the important originality of cultural transmission it is necessary to visualise several such diagrams, each representing a different family line (Figure 20). The consequence of this property is that a new characteristic acquired by a family line can not only persist in that line but may also invade other lines in the population. As several studies, have shown, this property can profoundly change the fate and evolutionary dynamics of populations.

Arrow 6 represents more than cultural transmission

Arrow 6 in Figure 19 also represents a range of vertical inheritance pathways from parent to offspring that were not discussed in the second part of this book, but which nonetheless have the potential to participate in non-genetic inheritance.

Microbiote transmission

A major discovery made possible by the advent of high-throughput sequencing at the turn of the third millennium is the importance of the microbiote, i.e. all the microflora without which we would be unable to live. The microbiote develops on the surface of our body and in our gut. It is often claimed that the number of microorganisms in our microbiote is about 10 times the number of cells in our body. However, a more recent estimate leads to a ratio of the number of microorganisms of the order of 1.3 times the number of cells in our

body, without taking into account viruses and other phages that may be in very large numbers given their tiny size[312].

Although this microbiote seems to fulfil functions that are absolutely vital for our survival, it is not this aspect that interests us here but rather the fact that in mammals, this microbiote is transmitted by the mother in two different ways at the time of birth. First, during natural birth, the baby ingests bacteria from its mother. Second, the mother's first milk, called colostrum, contains a whole host of molecules such as antibodies to protect the baby against external aggression, or antioxidants, or proteins and lymphocytes. But the colostrum also contains a whole microflora that the mother transfers to her offspring, inseminating it with the microflora that was so positive to her that she is raising a child. We will see in **Chapter 19** that the transmission or non-transmission of this microflora can affect development and participate in transmitting certain diseases.

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The transmission of prions and chaperone molecules

Another category of molecular memory transmitted along arrow 6 concerns the potential transmission of prions and chaperone molecules. In Erreur ! Source du renvoi introuvable., we discussed the importance of the 3D structure of proteins. This 3D structure is acquired by various folding of the amino acid chain that makes up the protein. Prions are proteins that for various reasons have not folded in the right way so that they no longer fulfil their metabolic function. These misconfigured proteins also have the ability to reconfigure other proteins of the same amino acid sequence into the same configuration as themselves, leading to a true contagion of this configuration and the accumulation of these dysfunctional proteins that can lead to spongiform encephalopathies such as scrapie, Creutzfeldt-Jakob disease or mad cow disease, as well as rare diseases such as fatal familial insomnia and Gerstmann-Straüssler-Scheinker syndrome. The emergence of prions seems to be strongly influenced by environmental stresses (such as temperature, osmotic or oxidative stress). In fact, to date relatively little is known about the role of prions in inheritance, with the exception of yeast[313]. My hunch is that, given the high stability and contagiousness of prions, we should expect them to produce original patterns of transmission (e.g. along a food chain), giving them a very special role in inheritance in inheritance[314]. Several authors, in view of the great transmission capacity of prions, include them in epigenetics.

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Chaperone molecules are somewhat the antithesis of prions in that they are molecules whose function is to assist newly synthesised proteins to fold in a functional manner, and then to maintain a wide variety of proteins in their functional configuration. One of the original characteristics of chaperone molecules is that they can act on a wide variety of proteins[315]. If these chaperone molecules are transmitted at all, it must be essentially vertically, and arrow 6 in **Figure 19** also would represents their effect in terms of intergenerational transmission.

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Cytoplasmic inheritance

Another area potentially capable of participating in parent-offspring resemblance is that of cytoplasmic inheritance. There are wonderful examples of this type of inheritance in single-celled organisms[316]. However, there is no reason to believe that this inheritance capacity was lost during the transition to multicellularity, where this type of inheritance could be involved in cell differentiation and thus in development. To my knowledge, this is an area that has not yet been explored very much.

Arrow 6 thus appears to represent several types of inheritance mechanisms of a highly diverse nature.

Ecological inheritance (arrow 7)

Ecological inheritance is often considered a marginal phenomenon, but it depends on what we considers as relevant to this phenomenon. If it is only a case of offspring inheriting the territory of their parents, then it is indeed relatively marginal in most species. However, even if it was marginal that would not mean that it does not affect evolution. Furthermore, it happens that ecological inheritance is far more common than the inheritance of parental territory.

Habitat imprinting…

After all, ecological inheritance emerges as soon as young individuals become imprinted on their birth habitat, leading them to seek to reproduce in the same type of habitat when they become adults. This well-known phenomenon called habitat imprinting is very common and well documented in birds and humans, for example. In forest passerines such as various titmice, for example, individuals may live either in deciduous or in evergreen forests. Young birds born in one type of habitat will show a strong preference for that type of habitat once adults. Similarly, in the American Cliff Swallow (*Petrochelidon pyrrhonota*) that nests in colonies ranging in size from 1 to over 3,000 breeding pairs, once they become adults the young tend to breed in colonies of the same size as the one in which they were born[317]. In humans, it is well known that whether we grew up

in a city or in the countryside has a strong influence on our adult preferences. Young adults born in a city often do not even imagine living in the country, and *vice versa*[318].

… as a force for divergence and diversity

The immediate consequence of habitat imprinting is that for generations individuals in a family line will be subject to the same selection pressure exerted by the type of environment in which different generations tend to settle. If we return to the case of the contrast between town and countryside in humans, there is no need to explain how the skills and abilities that are favoured in these two types of habitat are contrasted. It is therefore conceivable that if generations manage to settle in the same type of habitat for a long time, this could eventually lead to the selection of variants that, over time, could become so distant that individuals from different habitats never meet again or become incompatible for one reason or another. We would then have entered a diversification process called speciation, i.e. the genesis of two independent species.

Hence, ecological inheritance is probably much more common in nature than we think and can potentially play a significant role in evolution.

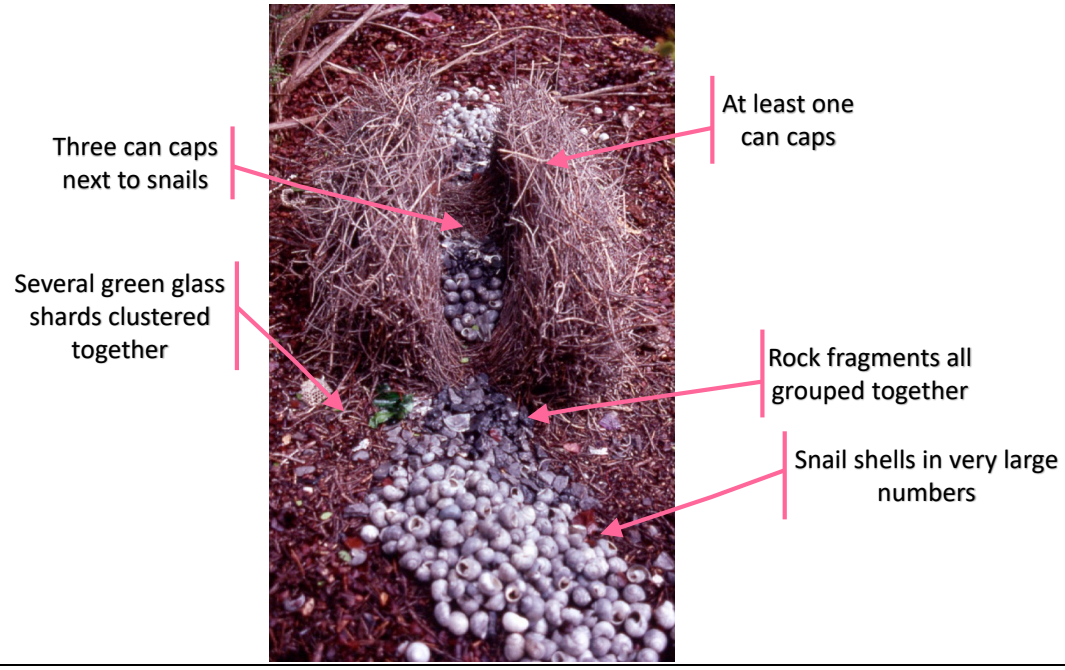
Niche construction (arrow 8)

Niche construction (discussed in **Chapter 7**) is often confused with ecological inheritance. However, these are quite different processes, but that often occur jointly. In many species that cannot move, offspring develop in the same place as their parents. This is the case, for example, in plants, or in single-celled organisms and bacteria. Therefore, all environmental changes made by individuals during their lifetime will be *de facto* inherited by their offspring[319]. For example, the secretion of acidic metabolic waste into the environment will necessarily acidify the area. Over several generations this effect can lead to a slow but inexorable change in environmental constraints acting on future generations who will have to cope with this accumulation of acidity in the environment. As another example, beavers build dams that persist beyond the lifetime of the builders and are often used and maintained by their offspring. Similarly, in bower birds, males build an elaborate structure called a bower to attract females, decorating them with anything bright and colourful in their environment. I had the opportunity to observe one in Australia decorated with shards of glass, and numerous Coca-Cola can caps (**Figure 21**). It was a relatively moderate size structure, about 50 cm high, but considering that these birds are the size of a large blackbird, this would be the equivalent of a multi-storey structure for a human.

Two points need to be made about bower birds. Firstly, it seems that males have different tastes, some preferring objects of a given colour. It would be interesting to investigate whether this might lead to the differentiation of sub-populations with different colour preferences, similar to what we saw in *Drosophila* in **Chapter 11**. On the other hand, these bowers outlive their builder and are taken over by another male when they are no longer in use, each male contributing to the increase in size of the structure, some might reach up to three metres in height[320]. This is probably a case of niche construction in the context of the mate choice, one of the most crucial decisions of any sexually reproducing organism. Second, human activities participate in a niche construction, which can considerably modify the environment in which we live, with the effect of favouring traits that may be significantly different from those favoured a few generations earlier.

But the phenomenon does not end there, as some of the phenotypic and behavioural responses developed in response to environmental change can be passed on to subsequent generations, beyond the persistence of the environmental stress. This raises a fundamental question. Why are some plastic responses to environmental stresses passed on while others are not? We have seen that an answer to this recurring question is that only the transmission of responses to environmental changes that persist beyond a single generation should provide an evolutionary advantage. It is clear that the phenomenon of niche construction is one of them.

Figure 21: The bower of a great bower bird (*Chlamydera nuchlis*) in Australia. The bower has the form of a corridor between two walls made of densely interwoven twigs. In this corridor one can see at least three capsules of industrial cans, as well as numerous decorative objects grouped by type[321].



What are the limits of niche construction?

In this book, I adopt a rather narrow definition of Niche construction, although several tenants of the EES tend to incorporate into that concept all forms of non-genetic inheritance[322]. I do not see the heuristics of broadening the field of niche construction beyond its natural, and already very important natural scope. As a consequence these two disciplines tend to ignore each other, which weakens the overall momentum for the emergence of the new synthesis.

As I develop here, I think that we should rather stress the central role of inheritance in evolution because this allows the transposition of a lot of methodologies across disciplines in order to link infra- with supra-individual biology. This is, for instance, what we did in Chapter 11, where adopting tools and concepts from quantitative genetics allowed us to develop a new definition of culture that can be transposed to any kind of organism. Furthermore, it seems to me that the links between niche construction and evolution are far less direct than the one between inheritance and evolution, because heredity is at the heart of evolution by natural selection or drift. Traits can evolve only if they are inherited (as Darwin said).

Epigenetically-facilitated mutational assimilation (arrow 9)

We developed the rationale for arrow 9 in Chapter 10. In particular, we saw that this process takes place over many (possibly thousands) generations. This is why the corresponding arrow is dotted.

What we have proposed with this mechanism is that when an environmental change happens to persist for more than one generation it would be advantageous to encode the information on how to cope with it in an inclusively heritable way that is nonetheless not too durable. But, if that change appears to persist for longer and longer periods of time, it becomes advantageous to encode it step by step in increasingly durable ways as the change persists over a growing number of generations. If this number of generations becomes very large, then, indeed, it would be advantageous to engrave it in the DNA sequence, i.e. in an irreversible way. It should therefore not be surprising that the as yet hypothetical process of epigenetically-facilitated mutational assimilation that we have proposed is slow, as this slowness is integral to its adaptive power.

You will have noticed that I am using the conditional tense here because all this remains to be explored. We have only proposed, on the basis of reasoning based on well-demonstrated facts, an idea that is still speculative and that some may consider iconoclastic. Only the future will tell whether we were right to anticipate such a phenomenon.

The environment as a factor of inheritance (arrow 10)

The environment is the starting point for all the pathways of non-genetic transmission across generations represented by the various red arrows in Figure 19. The effects of the environment on the phenotype are most likely all mediated by changes in epigenetic state. This is why arrow 10 does not link the environment directly to the phenotype but rather to gene expression[323]. Thus, the environment is an important player in inheritance because, as we saw in detail in the second part of this book, some environment effects can then be transmitted over many generations. This is why arrow 10 is green at first (to mark its role in phenotypic plasticity) and

then turns red (to mark the fact that some environmental effects can be inherited). We will return to the mechanisms underlying this important arrow in the next chapter.

Conclusion: a pluralistic view of inheritance

Inheritance thus passes through a whole series of pathways that are infinitely more subtle and complex (**Figure 19**) than what we classically think of with the purely sequencic vision of life called the Modern Synthesis of Evolution, itself represented in Erreur ! Source du renvoi introuvable.**B**. We shall see in **Two recurrent** reactions are that all these discoveries are certainly interesting, but they do not change much in the way evolution works, and all these processes are already taken into account. This attitude is like the one we talked about earlier, which consists of clinging to the old model to avoid having to change things too much. As we have seen, this amounts to denying the concept of emergent properties, which states that the properties of the whole entity somehow escape the properties of its components. Such reactions are understandable, and they force the proponents of the emergent view to sharpen their arguments and to bring new facts showing the strength of their conception in order to make it indisputable and unavoidable. In this last section, I will not discuss whether the Inclusive Evolutionary Synthesis brings fundamental changes, because as we have seen it is still far too early to answer this question. Instead, I will illustrate how taking into account non-genetic inheritance allows us to make progress in the understanding of a series of points, of evolutionary, conservation or medical relevance to develop immediate applications for the sustainable functioning of human societies. Chapter 18 to **Chapter** 20 the extent to which this can change the way evolution works, but also the way we search for solutions to various medical or conservation problems.

Therefore, by integrating into the classical diagram of Erreur ! Source du renvoi introuvable.**B** the numerous transmission pathways involved in transmitted resemblance, ***Figure 19*** *constitutes a fourth integration step from the Modern Synthesis of Evolution towards the Inclusive Evolutionary Synthesis.*

Chapter 16
Evolving Neo-Darwinism to rejuvenate Darwinism

In his famous book "*The Selfish Gene*", *Richard Dawkins* emphasised the fundamentally "selfish" nature of the gene replicator[324]. Here, the term "selfish" sanctions the fact that genes that, because of their action on the phenotype that carries them, manage to duplicate faster than the others are mechanically (and not intentionally) favoured by natural selection. They will therefore increase in proportion in the population, which can only occur *at the expense of* the other genes. This characteristic is perfectly captured by the word selfish, which in fact condenses *Herbert Spencer*'s famous phrase "The survival of the fittest"[325]. Genes whose actions do not lead the individuals carrying them to reproduce at the highest rates in their population are condemned to disappear from the population in the long run, if of course the local environmental conditions remain unchanged. This property, which is inexorable and devoid of any value judgement, is perfectly encapsulated in the term selfish.

This fundamental idea, which was clearly already in the air in the 1970s, was very structuring because it opened up a debate on the question of the entities that are selected. This debate is still ongoing, and will remain so for a long time because the answer to this question probably depends on the context.

How the inclusive view of inheritance does differ from Dawkins' view?

I had read The Selfish Gene in the 1980s and had only a vague memory of it. I especially remembered his Chapter 11 on the possible existence of other replicators, which was then the last chapter. It was that memory that led me to place The Selfish Gene at the centre of my thinking, because the Inclusive Evolutionary Synthesis in effect integrates the existence of a series of selfish pseudo-replicators that, together with the genetic (sequencic) replicator, participate in transmitted resemblance. I re-read Dawkins book after finishing the first draft of this one. I was struck by the fact that 95% of what *Richard Dawkins* wrote nearly 50 years ago remains entirely valid within the framework of the Inclusive Evolutionary Synthesis. This shows how the latter does not in any way challenge the Modern Synthesis of Evolution. The Inclusive Evolutionary Synthesis is an outgrowth of the Modern Synthesis of Evolution that provides a solid foundation on which to build a more general and inclusive vision.

The "5%" I disagree with concerns two important aspects of the Modern Synthesis that are worth discussing.

What is a gene?

In fact, Dawkins does not clearly distinguish between the pre- and post-DNA meanings of the gene concept. If he stuck to his definition in Chapter 1 as "the unit of heredity", then this aspect of my disagreement would disappear. This definition is undoubtedly pre-DNA, based on a statistical measurement of parent-offspring resemblance, and is therefore open to all forms of transmitted resemblance. It fits perfectly with what I call inclusive heritability because it has no mechanistic undertones.

However, later on, in his Chapter 3 —and implicitly throughout the rest of the book— Dawkins clarifies the definition of this concept in a purely molecular and therefore post-DNA sense. So the gene is defined as a portion of a chromosome, i.e. as a portion of DNA, or as "*A replicator with high fidelity*". Further on, "*a gene has to be a portion of a chromosome. The question is, how big a portion...*". It should be noted that these quotes do not insist on the DNA sequence, but a good part of that chapter is then devoted to describing the sequencic structure of DNA and the resulting genetic code and protein synthesis[326]. According to Jarvid Ågren, who produced a magisterial review on the Gene's-eye view of evolution (i.e. the Modern Synthesis), Dawkins' defines a gene as any part of a chromosome that is not broken up by recombination and that is therefore passed on intact across generations[327].

Apart from the fact that this is a typical example of the ambiguous use made by even the greatest authors of the two main definitions of the gene, it is clear that if I apply this second definition to the whole book — and this is undoubtedly how *Richard Dawkins* understands it[328]— then this reduces parent-offspring resemblance to the transmission of DNA sequencic information alone. This is the only real point of

disagreement between his view and the one I am defending here. This is both a small and an important point, because it ignores all the forms of transmitted resemblance developed in the second part of this book.

The origin of variation

My second point of disagreement is more implicit than explicit, because Dawkins does not clearly address the question of the origin of the variation on which selection can act. In general, the question of the origin of variation is too often evaded by evolutionists thanks to two authoritative arguments that are rarely discussed. First, as we have seen, the Modern Synthesis postulates that mutations are random —more precisely, not directed by the environment in the sense of adaptation. On the other hand, authors often postulate the pre-existence of hidden and initially neutral standing sequencic variation that is revealed in particular contexts following changes in selection pressure. In my opinion, these are two assertions that deserve to be nuanced.

Concerning the first point, we saw in Erreur ! Source du renvoi introuvable. that this is one of the basic principles of Neo-Darwinism that is often taken too far by asserting that 'mutations are random'. In particular, we have seen **in Chapter 10** that it would be appropriate to revisit this general principle of the Modern Synthesis that mutations are totally random with respect to the environment. The mechanism we have proposed, if it were to exist, would facilitate the emergence of variation, admittedly randomly, but specifically in the parts of the genome relevant to the adaptation to the concerned environmental challenge. This is what we called "epigenetically-facilitated mutational assimilation". If this mechanism, which we predicted and modelled on the basis of sound biological data, were to exist, it would lead us to temper the classical formulation of this principle[329].

Concerning the pre-existence of standing genetic variation, it seems difficult to believe that there is always some hidden variation ready to be opened to selection when conditions require it[330], and it seems even more difficult to imagine that this hidden variation is sufficiently diverse so that it always contains variants that will turn out to provide a selective advantage following all the environmental changes that may occur. Taken to the extreme, this vision could be viewed as reminiscent of a teleological conception that would be incompatible with the main principles of the Modern Synthesis itself.

But again, it is more the lack of discussion of this point in The Selfish Gene that disturbed me as it maintains some unnecessary ambiguity. So it's more a reaction to the absence of discussion on that point than to actual statements in the book.

Multiple selfish pseudo-replicators…

With the exception of these touchy points, the vision I develop in this book does not in any way challenge the fact that every replicating entity is *de facto* selfish, quite the contrary. In fact, the last chapter of The Selfish Gene did the intellectual and then purely abstract exercise of imagining the existence of other replicating entities. Dawkins wrote some absolutely visionary sentences. For example, on page 191 of the 2006 edition in which he did not change the original text but only added commentary notes at the end of the book, he writes sentences that I would be proud to have written myself: "…*for understanding evolution, we must begin by throwing out the gene as the sole basis of our ideas on evolution. I am an enthusiastic Darwinian, but I think Darwinism is too big a theory to be confined to the narrow context of the gene.*"[331] I couldn't say it better, and in fact my book takes him at his word.

At that time, he had rightly thought of the possible pseudo-replicator that would be culturally transmitted information. His Chapter 11 develops the concept of meme (pronounced as in gene). That chapter, which was originally a kind of provocation, in fact gave birth to what is called memetics (the science of the meme) which is still very much alive today[332]. But we saw in **Chapter 10 Randomness and mutation**

After discovering all these fascinating pathways of intergenerational information transfer, it is now necessary to develop an overlooked but basic property of epigenetic marks that is linked to a recurring issue in evolutionary biology, namely that of the randomness of mutations of all types. We have seen that one of the basic principles of the Modern Synthesis is that mutations are in no way directed by the environment towards improving the adaptation of organisms. Unfortunately, this principle is often simplified into saying that mutations occur at random, which does not mean the same thing. But what exactly is the case? This is what we will look at in this chapter.

Epigenetic marks are mutagenic…

The starting point that led me to think about the issue of mutation randomness was the fact that epigenetic marks, such as the presence of methyl radicals on cytosines, destabilises DNA and greatly increases the mutation rate of methyl-cytosines into thymine, another base of the DNA sequence. This, therefore, has the potential to generate point mutations whereby a cytosine is replaced by a thymine. Some articles have, for example, subheadings entitled "Methylation is mutagenic". For example, studies in humans suggest that

cytosine methylation is responsible for 30-40% of point mutations in the human germline. Combining the results of several authors, cytosine methylation would increase the probability of cytosine mutating to thymine by a factor of about 20,000. This is such a considerable factor that it seems very unlikely that it is a negative collateral effect of a process selected in another context (in this case DNA methylation, which is involved in the regulation of gene expression). What then could be the function of a process that destabilises the fidelity of sequencic transmission to such an extent?

This is what we addressed in a 2019 paper. We proposed a mechanism by which such mutagenic power of DNA methylation, and more generally of epigenetic marks, might have provided a real evolutionary advantage by accelerating the sequencic engraving of the initially plastic responses to environmental conditions that prove to be very persistent. We have given this mechanism the explicit but unmemorable name of *epigenetically-facilitated mutational assimilation*.

Genetic assimilation

The idea of *genetic assimilation* (see Glossary) was proposed by *Conrad Waddington* following a series of experiments in *Drosophila* showing that following an environmental stress triggering an initially plastic response, this response tends to become heritable (and therefore non-plastic) after a certain number of generations under the effect of this stress. It was therefore as if, after a few dozen generations, characters initially developed in a plastic manner in response to a given environment became ‘genetically’ engraved, hence the expression 'genetic assimilation'.

Genetic or epigenetic assimilation?

However, it should be noted that in this expression the term genetic was understood in its pre-DNA sense, as 'that which is transmitted', without prejudging the mechanism responsible for this transmission. In particular, while Waddington's experiments undoubtedly demonstrated that the initially plastic trait became inclusively heritable, they did not at all show that this necessarily implied a sequencic change. In effect, there was nothing in these experiments to suggest that what he observed at the phenotypic level resulted from a change in the DNA sequence. Given that Waddington had only worked over a few dozen generations —which was already a real challenge —he in fact most likely documented an "epigenetic assimilation" because the only thing his experiments really showed was that an initially plastic trait became inclusively inheritable within a few generations. This is equivalent to what *Mary Jane West-Eberhard* called "genetic accommodation" whereby a trait can be made heritable without necessarily involving encoding in the DNA sequence. Our paper proposed that, under certain conditions to which we will return later in this chapter, this process could go as far as sequencic engraving, *if the environmental stress persists over many, many generations*.

And the Modern Synthesis assimilated genetic assimilation

It has always puzzled me that the idea of genetic assimilation has finally been 'assimilated' by the Modern Synthesis, as this mechanism is strongly reminiscent of the much-rejected idea of inheritance of acquired traits. If you think about it, Waddington's mechanism proposes that within a few dozen generations under a given constant environmental stress the initially plastic response to stress can become heritable. In fact, what has allowed the idea of genetic assimilation to be assimilated is the relative slowness of this phenomenon. Moreover, the classical interpretation of this phenomenon is that there would pre-exist some neutral and hidden sequencic variation (usually called standing genetic variation) that would be somehow revealed by the environmental stress. Natural selection would then have the time to act over the few dozen generations of Waddington's experiments to retain only those variants that happen to be, I would like to say ‘miraculously’, favourable. So genetic assimilation would be just a special case of natural selection. This is how the Modern Synthesis has managed to see no major contradiction in genetic assimilation. This is also how I understood it until a few years ago.

Epigenetics as a hub towards sequencic engraving

A striking result on which we have built our reasoning is that all mechanisms of non-genetic heritability seem to involve some epigenetic change. It is as if epigenetics was the backbone or hub towards which most non-genetic inheritance processes would converge. Then, as epigenetic marks destabilize the DNA, over the course of many generations, this would generate sequencic variation *in the parts of the DNA concerned by the accommodation to the environmental change*. This would lead through natural selection acting on this newly produced variation, to sequencic engraving. In a way, epigenetics would be the conductor of the orchestra made up of all the genetic information. In effect, while it is very useful to have all the sequencic information (the recipe book), it is important to use it wisely. We shall see in **Chapter 16** that this epigenetic conductor is itself under the control of the brain.

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With *Arnaud Pocheville*, then based at the University of Sydney in Australia, we modelled this idea and were able to show that such a mechanism could accelerate the transfer of epigenetic encoding to sequencic encoding by a factor of the order of magnitude of the mutagenicity of the epigenetic marks, i.e. about 20,000 times. *This is what we called the epigenetically-facilitated mutational assimilation*.

But the story does not end there, as epigenetics interacts strongly with another major source of mutation, namely transposable elements.

... and interact with transposable elements

In parallel, we have been interested in another major phenomenon that can affect both the expression of certain genes and the appearance of mutations of all types. In fact, not only can the presence of epigenetic marks affect the stability of DNA, but epigenetic marks are themselves in strong interaction with the activity of transposable elements. Transposable elements are mobile DNA sequences discovered in maize by *Barbara McClintock* at the Cold Spring Harbor Laboratory on Long Island in the USA in the 1940s. This is one of the great genetic discoveries of the second half of the 20th century. There are a variety of transposable elements that differ, among other things, in the way they duplicate. Transposable elements exist in almost all living organisms. They seem to be able to invade the genome of an entire species through a process of colonisation from a local population, and can represent a large portion of the genome (about 15 to 22% in Drosophila, 40% of the genome in humans, and up to 90% in wheat). To give an idea of the prevalence of transposable elements, in humans, more than three million human sequences are derived from transposable elements, but only a few hundred of these have retained transposition capacity. The universality and mobility of transposable elements suggest that they play an important role in genome evolution and plasticity

The activity of transposable elements is under epigenetic control

The activity of transposable elements is strongly modulated by epigenetic processes (involving methylation, histone modifications or small RNAs) which are themselves affected by environmental factors. There are several hypotheses (not necessarily mutually exclusive) explaining the interaction between transposable elements and epigenetics. In particular, the targeting of epigenetic modifications to transposable elements could be a consequence of the *exaptation* (see Glossary) of transposable elements as platforms for chromatin modification, in which case the epigenetic regulation of transposable elements could be a consequence of genome defence and regulation. As a result, environmental stresses can trigger transposition activity, either directly or through their effects on epigenetic marks associated with transposable elements. It can be said that in most cases the mobility of transposable elements is inhibited by epigenetic marks that block their replication. However, this targeting of epigenetic marks on transposable elements also affects, as if by ricochet, the genes close to these transposable elements —with which they become partners in a kind of "transposable-element-gene duo"—, thus affecting their expression level. Beyond their important mutational effects, by duplicating themselves in the genome, transposable elements can thus affect the general functioning of the genome, among other things by regulating and controlling the activity of genes in the neighbourhood of their insertion point. Thus transposable elements affect gene activity in three different ways.

- First, by attracting strong epigenetic marking around their insertion point, they affect the epigenetic marks, and therefore the expression, of the genes with which they are in duo. It should be noted that the epigenetic marks around transposable elements can be modified by stresses bringing back their mobility, hence modifying the expression of the genes around the new insertion point.
- On the other hand, as the sequence of many transposable elements carries regulatory elements of response to the environment, their presence will directly modulate the expression of the genes with which they are in duo according to the environmental context. They therefore play a central role in the response to environmental changes.
- Finally, by their mobility within the genome, transposable elements can generate significant sequencic changes in the genome. Their mutagenic potential is thought to increase the average point mutation rate by several tens of thousands of times.

A great generator of inclusively heritable variation

Thus, the presence of transposable elements in one area of the genome can on the one hand durably modify the expression of the surrounding genes due to the strong intervention of persistent epigenetic marks inhibiting their mobility, and on the other hand generate genetic (sequencic) variation in the whole genome as a result of their mobility. Both types of variation can affect the phenotype either negatively for individuals (e.g. they are implicated in various diseases) or positively at the population level by generating variation that is inclusively heritable and therefore open to selection. In other words, while at the individual level these changes can often have negative consequences, at the population level transposable elements generate inclusively heritable variation on which natural selection can act, thus favouring the adaptation of populations to their environment.

Interactions between epigenetics and transposable elements thus constitute a real engine for the creation of phenotypic variation (targeted to specific portions of the genome) that can be inherited either sequentially or epigenetically *in response to environmental stresses*, and are thus an important factor in evolution. Such a generator of genetic and epigenetic variation can in particular explain changes in mutability within the genome following environmental stresses. Several authors have emphasised the existence and importance of such generators of inclusively heritable variation involving the joint action of genetic and non-genetic processes in the ability of natural populations to adapt to ongoing global changes under the influence of human activities.

Epigenetically-facilitated mutational assimilation

We can now synthesize this. It appears that the effects of environmental stresses can affect the expression of specific genes involved in the response to stress and affect the activity of transposable elements, two major characteristics that each have the capacity to increase the sequencic mutation rate by tens of thousands of times, which is anything but negligible.

An information transfer pathway acting over many generations

The epigenetic changes affecting the expression of genes specifically involved in the response to an environmental stress in fact have two functions taking place on two very different time scale:

- First, these epigenetic marks, which we have seen target very precise portions of the DNA, enable the individual to adapt to the current environment by finely regulating the expression of the genes involved and leading to the phenotypic response to the environmental challenge. This response is rapidly established under the effect of environmental change. This process is known as phenotypic plasticity, the ability to modify the phenotype in response to the environment.
- Second, by being inherited, those epigenetic marks lastingly affect the mutability of the concerned genes that happen to be the genes involved in the accommodation to the specific environmental change. These epigenetic marks can also affect the activity of neighbouring transposable elements, which can further increase the mutability of the concerned regions and thus the potential generation of sequencic variation. In other words, epigenetic marking would differentially mark portions of the genome for mutation, i.e. for the generation of sequencic variation and thus for the multigenerational exploration of new genetic possibilities. Far from being a cost in terms of evolution, this may on the contrary constitute a major evolutionary benefit because the sequencic variation thus generated concerns the genes actually involved in the accommodation to the specific environmental stress, a variation then open to natural selection.

This is *epigenetically-facilitated mutational assimilation* that is more than just a special case of natural selection on initially neutral and hidden genetic variation suddenly revealed by environmental change. According to our view, genetic assimilation appears as *a genuine mechanism for manufacturing sequencic variation in the parts of the genome concerned by the accommodation to the specific environment*, variation which is then open to natural selection. This mechanism calls for several important comments.

Random mutations in environmentally targeted areas of the genome

First, with epigenetically-facilitated mutational assimilation, the fundamental axiom of the Modern Synthesis that *mutations are not influenced by the environment in an adaptive direction* remains 100% valid. However, it is the simplified phrase traditionally used to simplify this axiom "mutations are random" that appears incorrect. With epigenetically-facilitated mutational assimilation the mutations generated following a lasting environmental change are indeed not influenced in an adaptive direction by the environment (the axiom of the Modern Synthesis therefore remains valid), but the parts of the genome where the mutation rate increases are actually targeted by the environment. *This is because epigenetic changes and the activity of transposable elements are themselves targeted by the environment*. There are therefore two independent scales where randomness can be expressed, that of regional portions of the DNA, and that of the local change of sequence itself. Only the second scale is unaffected by the environment, whereas the regional scale is clearly targeted by the effects of the environment in the sense that it is precisely in the portions of the DNA concerned by the accommodation to the environmental challenge that the mutation rate changes.

A necessarily slow process…

Second, even if the magnitude of several tens of thousands of increase in mutation rate seems enormous, it does not mean that epigenetically-facilitated mutational assimilation (i.e. the sequencic engraving of the adaptation) takes place in a few generations. A rough calculation predicts that such a process must take hundreds, if not thousands, of generations to become effective. Although the calculation proposed in the last note is very crude, the important point is that we should not expect epigenetically-facilitated mutational assimilation to take place very quickly, and certainly not in only a few tens of generations. And in fact, evolutionary logic even leads us to believe that this slowness is integral to the process (see below).

... which could be involved in domestication

We were certainly not the first to think about this type of genetic assimilation where the environment can be involved in generating genetic variation in the sections of the genome involved in the response to the environment. For example, one of the earliest papers on the subject dates back to 1983 in which *Hugh Iltis*, then Professor of Botany at the University of Wisconsin, formalised a scenario for the domestication of maize from teosinte, an annual plant from Central America. This remarkable scenario integrated several previous hypotheses and involved the major and massive effect of what he called a catastrophic epigenetic sexual transmutation that occurred some seven millennia ago.

Similarly, the whole literature on transposable elements claims that the environment can generate inclusively heritable variation. Regarding the idea that the environment can generate variation in certain regions of the genome, *Eva Jablonka* and her collaborators had modelled this idea without proposing a molecular mechanism. Similarly, *Michael Skinner* also foresaw and proposed the existence of such phenomena. Furthermore, researchers working on the domestication syndrome of vertebrates proposed that the stress induced at the beginning of domestication must have caused alterations in the methylation patterns of developmental genes expressed in the neural crest (the part of the embryo that will become the central nervous system), epigenetic changes that could have been fixed in the form of genetic variants to explain recurrent behavioural resemblances in the many domesticated fish, mammals and birds.

The different systems of inheritance interact with each other

This chapter thus introduced a particularly important point, namely that the different systems of inheritance (which we will summarise in **Chapter 15**) do not operate independently of each other. On the contrary, they interact and influence each other. For example, the central idea of epigenetically-facilitated mutational assimilation is that the molecular memory represented by epigenetics states interacts over the long term with sequencic memory, in a way that can potentially considerably accelerate the genetic encoding of initially plastic responses to environmental characteristics that persisted for hundreds or thousands of generations.

Chapter 11, that his intuition was even more well-founded than he thought at the time, because while he had mainly focused on the case of humans[333], we now know that the cultural phenomenon is not limited to humans alone and probably concerns a large number of species[334].

What is most surprising is that *Richard Dawkins*' book was later used to defend the idea that, although there may be other replicating entities than genes[335], genes are the only *true* replicator. This reductionist view does not do justice to the foresight of its author, and it is still dominant today and resurfaces whenever, in response to one of my lectures, someone says something like "Yes, but in the end it all comes down to genes". We saw in **Chapter 12** how inappropriate this statement is, and my aim is to convince people that recent findings show that there may be other types of selfish replicators (or replicating entities). If the reductionist view of just considering the genes replicator was a necessary and justified step in the 1950s to 1980s, today it becomes necessary to go beyond that by integrating the whole reality of non-genetic inheritance. It is true that genes are an indisputable key replicator, but today duly documented scientific facts suggest that the concept of replicator can be generalised to produce the concept of "pseudo-replicators" or "replicating entities" as we defined in **Chapter 12**. Thus, it would be foolish to continue to reduce the concept of replicators to the sole gene. We must come to accept that the gene replicator is acting jointly with other replicating entities in the functioning and perpetuation of life over generations and geological time. This chapter presents the fifth step in the construction of the framework necessary to achieve this integration, or synthesis.

... with contrasting properties and functions

Having said that, we should not want to transfer blindly all our knowledge on the gene replicator to the pseudo-replicators. We should not be surprised to discover the extent to which the properties of the non-genetic replicating entities differ from those of the gene replicator. It is those differences that give them their specific functions in inheritance. I regularly had to struggle against all my prejudices in this domain. Obviously I was not the only one to face that dilemma. For instance, that difficulty is evident when *Jarvid Ågren* discusses the meme concept[336], leading me to think that we should not try to transpose the rationale of the gene too much to pseudo-replicators. We must be more open-minded and be prepared to discover replicating entities with drastically different properties, fuzzier avatars, and following very different rules of transmission, as well as to have to accept a non-discreet nature of some replicating entities (a question which is already delicate concerning the discrete or continuous nature of the genetic replicator), etc.

There must be conflicts between different types of replicating entities

There is, however, an inevitable consequence of the existence of different types of replicating entities that I just want to mention here. This diversity of replicating entities must necessarily give rise to conflicts among them and with the gene replicator. The literature is full of examples of conflicts of interest between different

genes and even between copies of the same gene depending on whether they have been passed on from the father or the mother. It is therefore inevitable that such conflicts will emerge among genes and pseudo-replicators. However, as we are only at the stage of accepting the existence of many replicating entities of very different natures, there are so far very few empirical arguments for the existence of such conflicts. One argument that comes to mind is that put forward by *Kevin Laland*, now at Saint Andrews University in Scotland, who has reported evidence suggesting the existence of conflicts between the sequencic and the cultural pseudo-replicator[337]. Laland showed that in a context of mate choice, a culturally transmitted mating preference can draw a fitness-decreasing trait to fixation. His argument is that in this case a conflict between a cultural pseudo-replicator and a genetic replicator would be won by the cultural pseudo-replicator. But really, my message on this topic is that this is an almost unexplored area that should emerge soon.

A generic mechanism for the effect of the environment on the phenotype

Although we have long known that the environment affects the phenotype, we have only partial information on the mechanisms by which the environment can influence gene expression in various parts of the organism, thus affecting the phenotype in ways that usually facilitate the accommodation of the organism to the relevant environment. Specifically, although there is evidence that, for example, epigenetic marks can be strongly affected by environmental stresses[338], we do not know how this actually occurs, nor do we know how environmental information is perceived, let alone how it is transmitted to cells to trigger these changes in gene expression. In other words, we do not have a generic process to explain how the environment can concretely affect gene expression along arrow 10 of **Figure 19**. This is, however, a fundamental element for understanding accommodation through phenotypic plasticity and for understanding the many examples of inheritance of responses to environmental changes such as those we have developed in Erreur ! Source du renvoi introuvable. to **Chapter 9**. The aim of this section is to propose such an integrative generic process[339].

Small non-coding RNAs (sncRNAs) as a long-ignored molecule of inheritance

First of all, we saw in Erreur ! Source du renvoi introuvable. to **Chapter** 10 Randomness and mutation

After discovering all these fascinating pathways of intergenerational information transfer, it is now necessary to develop an overlooked but basic property of epigenetic marks that is linked to a recurring issue in evolutionary biology, namely that of the randomness of mutations of all types. We have seen that one of the basic principles of the Modern Synthesis is that mutations are in no way directed by the environment towards improving the adaptation of organisms. Unfortunately, this principle is often simplified into saying that mutations occur at random, which does not mean the same thing. But what exactly is the case? This is what we will look at in this chapter.

Epigenetic marks are mutagenic...

The starting point that led me to think about the issue of mutation randomness was the fact that epigenetic marks, such as the presence of methyl radicals on cytosines, destabilises DNA and greatly increases the mutation rate of methyl-cytosines into thymine, another base of the DNA sequence. This, therefore, has the potential to generate point mutations whereby a cytosine is replaced by a thymine. Some articles have, for example, subheadings entitled "Methylation is mutagenic". For example, studies in humans suggest that cytosine methylation is responsible for 30-40% of point mutations in the human germline. Combining the results of several authors, cytosine methylation would increase the probability of cytosine mutating to thymine by a factor of about 20,000. This is such a considerable factor that it seems very unlikely that it is a negative collateral effect of a process selected in another context (in this case DNA methylation, which is involved in the regulation of gene expression). What then could be the function of a process that destabilises the fidelity of sequencic transmission to such an extent?

This is what we addressed in a 2019 paper. We proposed a mechanism by which such mutagenic power of DNA methylation, and more generally of epigenetic marks, might have provided a real evolutionary advantage by accelerating the sequencic engraving of the initially plastic responses to environmental conditions that prove to be very persistent. We have given this mechanism the explicit but unmemorable name of *epigenetically-facilitated mutational assimilation*.

Genetic assimilation

The idea of *genetic assimilation* (see Glossary) was proposed by *Conrad Waddington* following a series of experiments in *Drosophila* showing that following an environmental stress triggering an initially plastic response, this response tends to become heritable (and therefore non-plastic) after a certain number of generations under the effect of this stress. It was therefore as if, after a few dozen generations, characters

a sup
a sup
a sup
a mis
l'orth
a sup
a mis
l'orth
a mis

initially developed in a plastic manner in response to a given environment became ‘genetically’ engraved, hence the expression 'genetic assimilation'.

Genetic or epigenetic assimilation?

However, it should be noted that in this expression the term genetic was understood in its pre-DNA sense, as 'that which is transmitted', without prejudging the mechanism responsible for this transmission. In particular, while Waddington's experiments undoubtedly demonstrated that the initially plastic trait became inclusively heritable, they did not at all show that this necessarily implied a sequencic change. In effect, there was nothing in these experiments to suggest that what he observed at the phenotypic level resulted from a change in the DNA sequence. Given that Waddington had only worked over a few dozen generations —which was already a real challenge —he in fact most likely documented an "epigenetic assimilation" because the only thing his experiments really showed was that an initially plastic trait became inclusively inheritable within a few generations. This is equivalent to what *Mary Jane West-Eberhard* called "genetic accommodation" whereby a trait can be made heritable without necessarily involving encoding in the DNA sequence. Our paper proposed that, under certain conditions to which we will return later in this chapter, this process could go as far as sequencic engraving, *if the environmental stress persists over many, many generations*.

And the Modern Synthesis assimilated genetic assimilation

It has always puzzled me that the idea of genetic assimilation has finally been 'assimilated' by the Modern Synthesis, as this mechanism is strongly reminiscent of the much-rejected idea of inheritance of acquired traits. If you think about it, Waddington's mechanism proposes that within a few dozen generations under a given constant environmental stress the initially plastic response to stress can become heritable. In fact, what has allowed the idea of genetic assimilation to be assimilated is the relative slowness of this phenomenon. Moreover, the classical interpretation of this phenomenon is that there would pre-exist some neutral and hidden sequencic variation (usually called standing genetic variation) that would be somehow revealed by the environmental stress. Natural selection would then have the time to act over the few dozen generations of Waddington's experiments to retain only those variants that happen to be, I would like to say ‘miraculously’, favourable. So genetic assimilation would be just a special case of natural selection. This is how the Modern Synthesis has managed to see no major contradiction in genetic assimilation. This is also how I understood it until a few years ago.

Epigenetics as a hub towards sequencic engraving

A striking result on which we have built our reasoning is that all mechanisms of non-genetic heritability seem to involve some epigenetic change. It is as if epigenetics was the backbone or hub towards which most non-genetic inheritance processes would converge. Then, as epigenetic marks destabilize the DNA, over the course of many generations, this would generate sequencic variation *in the parts of the DNA concerned by the accommodation to the environmental change*. This would lead through natural selection acting on this newly produced variation, to sequencic engraving. In a way, epigenetics would be the conductor of the orchestra made up of all the genetic information. In effect, while it is very useful to have all the sequencic information (the recipe book), it is important to use it wisely. We shall see in **Chapter 16** that this epigenetic conductor is itself under the control of the brain.

a mis

With *Arnaud Pocheville*, then based at the University of Sydney in Australia, we modelled this idea and were able to show that such a mechanism could accelerate the transfer of epigenetic encoding to sequencic encoding by a factor of the order of magnitude of the mutagenicity of the epigenetic marks, i.e. about 20,000 times. *This is what we called the epigenetically-facilitated mutational assimilation*.

But the story does not end there, as epigenetics interacts strongly with another major source of mutation, namely transposable elements.

… and interact with transposable elements

In parallel, we have been interested in another major phenomenon that can affect both the expression of certain genes and the appearance of mutations of all types. In fact, not only can the presence of epigenetic marks affect the stability of DNA, but epigenetic marks are themselves in strong interaction with the activity of transposable elements. Transposable elements are mobile DNA sequences discovered in maize by *Barbara McClintock* at the Cold Spring Harbor Laboratory on Long Island in the USA in the 1940s. This is one of the great genetic discoveries of the second half of the 20th century. There are a variety of transposable elements that differ, among other things, in the way they duplicate. Transposable elements exist in almost all living organisms. They seem to be able to invade the genome of an entire species through a process of colonisation from a local population, and can represent a large portion of the genome (about 15 to 22% in Drosophila, 40% of the genome in humans, and up to 90% in wheat). To give an idea of the prevalence of transposable elements, in

humans, more than three million human sequences are derived from transposable elements, but only a few hundred of these have retained transposition capacity. The universality and mobility of transposable elements suggest that they play an important role in genome evolution and plasticity

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- First, by attracting strong epigenetic marking around their insertion point, they affect the epigenetic marks, and therefore the expression, of the genes with which they are in duo. It should be noted that the epigenetic marks around transposable elements can be modified by stresses bringing back their mobility, hence modifying the expression of the genes around the new insertion point.
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A great generator of inclusively heritable variation

Thus, the presence of transposable elements in one area of the genome can on the one hand durably modify the expression of the surrounding genes due to the strong intervention of persistent epigenetic marks inhibiting their mobility, and on the other hand generate genetic (sequencic) variation in the whole genome as a result of their mobility. Both types of variation can affect the phenotype either negatively for individuals (e.g. they are implicated in various diseases) or positively at the population level by generating variation that is inclusively heritable and therefore open to selection. In other words, while at the individual level these changes can often have negative consequences, at the population level transposable elements generate inclusively heritable variation on which natural selection can act, thus favouring the adaptation of populations to their environment.

Interactions between epigenetics and transposable elements thus constitute a real engine for the creation of phenotypic variation (targeted to specific portions of the genome) that can be inherited either sequentially or epigenetically *in response to environmental stresses*, and are thus an important factor in evolution. Such a generator of genetic and epigenetic variation can in particular explain changes in mutability within the genome following environmental stresses. Several authors have emphasised the existence and importance of such generators of inclusively heritable variation involving the joint action of genetic and non-genetic processes in the ability of natural populations to adapt to ongoing global changes under the influence of human activities.

Epigenetically-facilitated mutational assimilation

We can now synthesize this. It appears that the effects of environmental stresses can affect the expression of specific genes involved in the response to stress and affect the activity of transposable elements, two major characteristics that each have the capacity to increase the sequencic mutation rate by tens of thousands of times, which is anything but negligible.

An information transfer pathway acting over many generations

The epigenetic changes affecting the expression of genes specifically involved in the response to an environmental stress in fact have two functions taking place on two very different time scale:

- First, these epigenetic marks, which we have seen target very precise portions of the DNA, enable the individual to adapt to the current environment by finely regulating the expression of the genes involved and leading to the phenotypic response to the environmental challenge. This response is rapidly established under the effect of environmental change. This process is known as phenotypic plasticity, the ability to modify the phenotype in response to the environment.
- Second, by being inherited, those epigenetic marks lastingly affect the mutability of the concerned genes that happen to be the genes involved in the accommodation to the specific environmental change. These epigenetic marks can also affect the activity of neighbouring transposable elements, which can further increase the mutability of the concerned regions and thus the potential generation of sequencic variation. In other words, epigenetic marking would differentially mark portions of the genome for mutation, i.e. for the generation of sequencic variation and thus for the multigenerational exploration of new genetic possibilities. Far from being a cost in terms of evolution, this may on the contrary constitute a major evolutionary benefit because the sequencic variation thus generated concerns the genes actually involved in the accommodation to the specific environmental stress, a variation then open to natural selection.

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A necessarily slow process…

Second, even if the magnitude of several tens of thousands of increase in mutation rate seems enormous, it does not mean that epigenetically-facilitated mutational assimilation (i.e. the sequencic engraving of the adaptation) takes place in a few generations. A rough calculation predicts that such a process must take hundreds, if not thousands, of generations to become effective. Although the calculation proposed in the last note is very crude, the important point is that we should not expect epigenetically-facilitated mutational assimilation to take place very quickly, and certainly not in only a few tens of generations. And in fact, evolutionary logic even leads us to believe that this slowness is integral to the process (see below).

… which could be involved in domestication

We were certainly not the first to think about this type of genetic assimilation where the environment can be involved in generating genetic variation in the sections of the genome involved in the response to the environment. For example, one of the earliest papers on the subject dates back to 1983 in which *Hugh Iltis*, then Professor of Botany at the University of Wisconsin, formalised a scenario for the domestication of maize from teosinte, an annual plant from Central America. This remarkable scenario integrated several previous hypotheses and involved the major and massive effect of what he called a catastrophic epigenetic sexual transmutation that occurred some seven millennia ago.

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The different systems of inheritance interact with each other

This chapter thus introduced a particularly important point, namely that the different systems of inheritance (which we will summarise in **Chapter 15**) do not operate independently of each other. On the contrary, they interact and influence each other. For example, the central idea of epigenetically-facilitated mutational assimilation is that the molecular memory represented by epigenetics states interacts over the long term with sequencic memory, in a way that can potentially considerably accelerate the genetic encoding of initially plastic responses to environmental characteristics that persisted for hundreds or thousands of generations.

Chapter 11, that small non-coding RNAs (sncRNAs, see **Box 2**) are important mediators of information transmission across generations. Although these molecules participate in the encoding of information over much shorter periods of time than the encoding via the DNA sequence, they nonetheless have real transgenerational effects[340]. These sncRNAs can be produced in various somatic cells and then released and distributed systematically throughout the body where they can affect gene expression[341]. These sncRNAs meet all the criteria for a hormone, making them ideal candidates for mediating environmental effects.

Thus, the information collected by all the sensory systems converges towards the brain, which is the natural information gathering and processing centre (top of **Figure 22**). The brain then produces sncRNAs which are then released into the circulatory systems (usually carried in extracellular microvesicles to protect them from degradation) reaching cells where they affect gene expression of highly specific regions of the genome in the whole body[342]. These sncRNAs are then displayed over three contrasting timescales.

The intragenerational scale

When epigenetic changes occur in somatic cells, they allow the immediate accommodation to the environment (output 1 in **Figure 22**, and arrow 5 in **Figure 19**). This involves the fine differential regulation of gene expression in different parts of the organism and facilitates the development and life of the concerned individual in response to the environment. These intragenerational changes in gene expression can unfold at two different timescales. First in real time, over very short periods of time leading to the fine tuning of gene expression in response to rapidly changing environmental factors. Second, some of these changes in gene expression persist across mitosis and thus are part of epigenetics (as defined in **Chapter 5**) and make up the non-transmitted phenotypic variance (V_{NT} in **Figure 16**) enabling phenotypic plasticity. They provide advantages for all environmental changes occurring more frequently than the time of one generation.

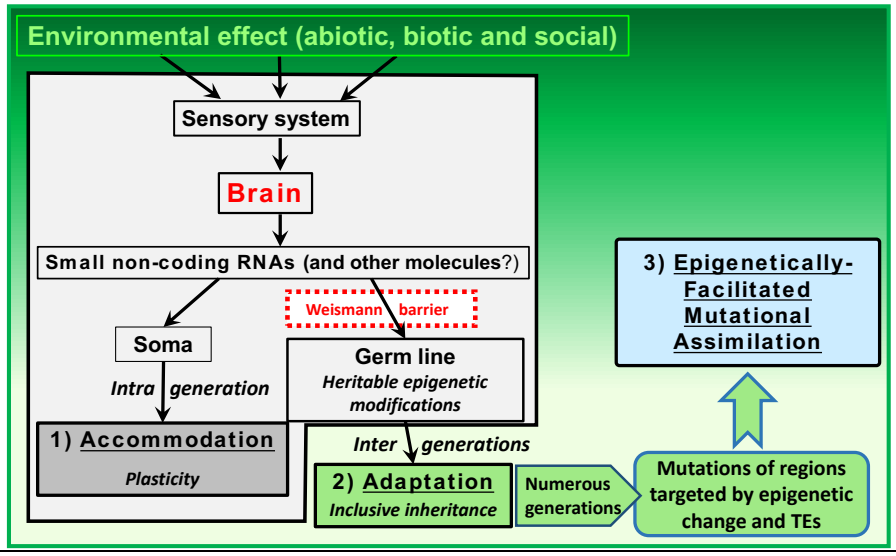
The intergenerational scale

When the epigenetic changes produced by the brain-born sncRNAs affect the germline, these changes are transmitted to the next generations (output 2 of **Figure 22**, and arrows 3 and 4 in **Figure 19**) producing the non-genetic inheritance of plastic responses that may persist over many generations[343]. The resulting heritable component participates to the adaptation of organisms to their environment through the selection on that variation. Such a mechanism only provides an evolutionary advantage in the face of environmental changes that will last for more than one generation.

Over numerous generations

Finally, if the environmental change persists over numerous generations (output 3 in **Figure** **Figure 22** and arrow 9 in **Figure 19**), this provides sufficient time for epigenetically-facilitated mutational assimilation to occur owing to transposable elements, and the mutagenicity of epigenetic marks (**Chapter 10**).

Figure 22: How the environment affects the phenotype. According to this integrative generic process 1) the environment can affect the phenotype, and such effects can 2) be transmitted, and 3) later lead to epigenetically-facilitated mutational assimilation through the joint effect of the mutagenicity of epigenetic marks and of transposable elements (**TEs**). This diagram describes the case of a brained organism, but all organisms have sensory systems informing them about environmental conditions and in organisms without a nervous system (plants or single-celled organisms), these processes can also involve small non-coding RNAs. For these organisms, simply remove the brain box and the diagram remains valid. I have chosen to leave in this diagram the possibility that other as yet unknown molecules are involved, although at present we have no indication of this and although small non-coding RNAs seem particularly suited to this role. Note that all the black arrows in this diagram are documented, the green arrows on the right-hand side being still partly speculative at this stage[344]. The white box surrounded with the red dotted line gives the position of the Weismann barrier. This concept needs some rethinking to integrate the fact that many processes can bypass it[345].



It is not known yet whether this effect occurs through the direct effect of brain-born sncRNAs on germ cells, or through the effect of these same sncRNAs on somatic cells that hence produce other sncRNAs that affect the epigenetic state of the gametes as we have seen in the transmission of some metabolic disorders (Chapter 9)[346].

Over a large number of generations, the epigenetically-facilitated mutational assimilation targets for mutation those parts of the DNA involved in the accommodation to the persistent environment. We saw in Chapter 10 that this mechanism would probably accelerate the sequencic engraving of the information by a factor equivalent to the mutagenicity introduced by the epigenetic marks, i.e. by a factor of 10^4, which is considerable. Output 3 in Figure 22 therefore details the molecular mechanisms underlying arrows 9 in Figure 19.

Consequently, this generic process operates at three very different timescales (ranging from that of immediate reaction to plasticity up to that of genetic adaptation) and suggests that there is a deep mechanistic link between development, accommodation and adaptation.

Small non-coding RNAs (see Box 2) as heirs to Darwin's gemmules

Darwin in the mid-19th century adhered to a view of inheritance called pangenesis, according to which, at the time of reproduction, hypothetical particles called gemmules were produced in all parts of the body and converged on the reproductive organs where they provided the information to reconstruct a complete individual. In fact, Figure 22 is strikingly reminiscent of this mechanism (that many, including myself until a few years ago, still consider to be fanciful). Indeed, in this figure, the sncRNAs play a role similar to that attributed to the gemmules of our predecessors. It shows that the history of science sometimes has surprising twists and turns.

The Weismann barrier revisited

Figure 22 suggests that the Weismann barrier, which is one of the foundations of the Modern Synthesis, needs some rethinking[347]. If there are processes that protect the germline from the effects of the environment, they are not 100% effective. We saw several examples in Chapter 7 to Chapter 9 of mechanisms of soma-germline communication that make it possible to bypass that barrier, to such an extent that one must question its very existence, in favour of the pangenesis of Darwin's contemporaries[348].

Moreover, the mechanism of epigenetically-facilitated mutational assimilation (see Chapter 10 and arrow 9 of Figure 19) also seem to challenge the Weismann barrier because according to this mechanism the environment may be involved in determining, through epigenetic marks, which themselves are precisely targeted by sncRNAs, the areas of the genome where mutations will preferentially occur. As we have seen, this implies that mutations are partly directed by the environment in the sense that the environment can target for mutation those portions of the genome that are concerned with the accommodation to the concerned environment. This is one of the implicit messages of Figure 22.

However, it could be argued that the mere fact that there are sophisticated DNA demethylation-remethylation mechanisms at meiosis and fertilisation shows that the Weismann barrier is partly permeable, as otherwise there would be no need to reset the epigenetic state of the information carried by the gametes. Nonetheless, the argument can be turned around by considering that the demethylation-remethylation waves at reproduction constitute the Weismann barrier, then it can be argued that this barrier does exist, but that it is

sometimes (though not rarely) bypassed by the sncRNAs —themselves under the influence of the brain integrating environmental information— added to the gametes during their maturation. Personally, I think that this last way of considering the existence of the Weismann barrier is probably the most correct, although I recognise that this is an unusual way of considering it.

The evolution of inheritance systems

In a lecture in 1932, the great statistician and geneticist *Ronald A. Fisher* stated that beyond genetic evolution there is also the science of genetic evolution studying how the major characteristics of genes (dominance, pleiotropy, mutations, etc.) change over time[349]. Transposed to the pluralistic vision of inheritance that I defend in this book, it implies that we must study the evolution of each of the systems of inheritance, remembering to take account of their interactions, and without assuming that this fundamentally challenges the Neo-Darwinian view.

The trade-off between transmission reversibility and fidelity

A common feature of all non-genetic inheritance systems is that heritable adaptation is not transmitted in a definitive and irreversible way as is the case with genetic transmission. Although this feature is often decried, from an adaptive point of view, this reversibility should instead be seen as a strength, allowing parents to mould the phenotype of their offspring to the currently prevailing environmental conditions, while leaving them the possibility of adopting another phenotype if the environment changes again.

Conversely, while the strength of sequencic inheritance lies in its very high fidelity of transmission, its lack of reversibility makes it inappropriate for adapting organisms to relatively rapid changes in the environment. This suggests that genetic and non-genetic inheritance, far from being antagonistic as is too often asserted, are complementary, each with rates of change corresponding to the various rates of environmental change[350].

It is the difference in rhythms of change of the various systems of inheritance that solves the fundamental compromise between needs at various timescales. On the one hand, it is necessary to have mechanisms for adapting to frequent changes in the environment that occur more frequently than the rate of generations. This is the function of phenotypic plasticity. Conversely, it is necessary to be able to store information in a highly faithful manner, allowing resemblance over the very long term. This is the role of sequencic inheritance. These two types of mechanisms are relatively well integrated in the Modern Synthesis.

However, in this view, as we began to discuss in **Erreur ! Source du renvoi introuvable.**, mechanisms are lacking to ensure resemblance at intermediate timescales, i.e. to ensure the transmission of ways of coping with changes that occur infrequently enough to persist over generations, but too often for genetic engraving to be adequate. This is the role of the various non-genetic inheritance systems, each acting over variable time windows (**Figure 15**). The opposition we made in **Chapter 12** between plasticity and heritability overlaps with the temporal cut-off between changes occurring more frequently or less frequently than the duration of one generation.

This reasoning leads to the somewhat provocative thought that it is probably the existence of non-genetic inheritance that has allowed genetic inheritance to become more and more faithful throughout the history of life, until it has reached the incredible levels of faithfulness of transmission that we know today[351].

Inheritance systems do not contradict each other, they complement each other

The existence of non-genetic inheritance is often presented as contradicting the importance of genetic inheritance. However, far from contradicting each other, the various inheritance systems are inherently complementary, with relative weights that vary among organisms depending on their own characteristics. For example, one can imagine that the inheritance of species living in variable environments rely more on non-genetic inheritance than species living in stable environments. Such a contrast exists globally between plants and animals. The former being sessile must adapt *in situ*, while the latter can buffer environmental variation by moving. In line with this ecological contrast, there is evidence that plants use significantly more non-genetic inheritance (which has a faster turnover) than animals, at least mammals[352]. Similarly, among animals, we might expect that inheritance in highly mobile taxa, such as birds and whales, rely more on sequencic than non-sequencic inheritance compared to much more sedentary groups that cannot homogenise their environment by moving[353]. In other words, we need to study the links between movement abilities and the relative weight of the genetic and non-genetic components of inheritance[354].

In conclusion, by integrating in a synthetic framework the recent results on the molecular mechanisms of non-genetic inheritance, ***Figure 22*** *provides a fifth step of integration from the Modern Synthesis towards the Inclusive Evolutionary Synthesis.*

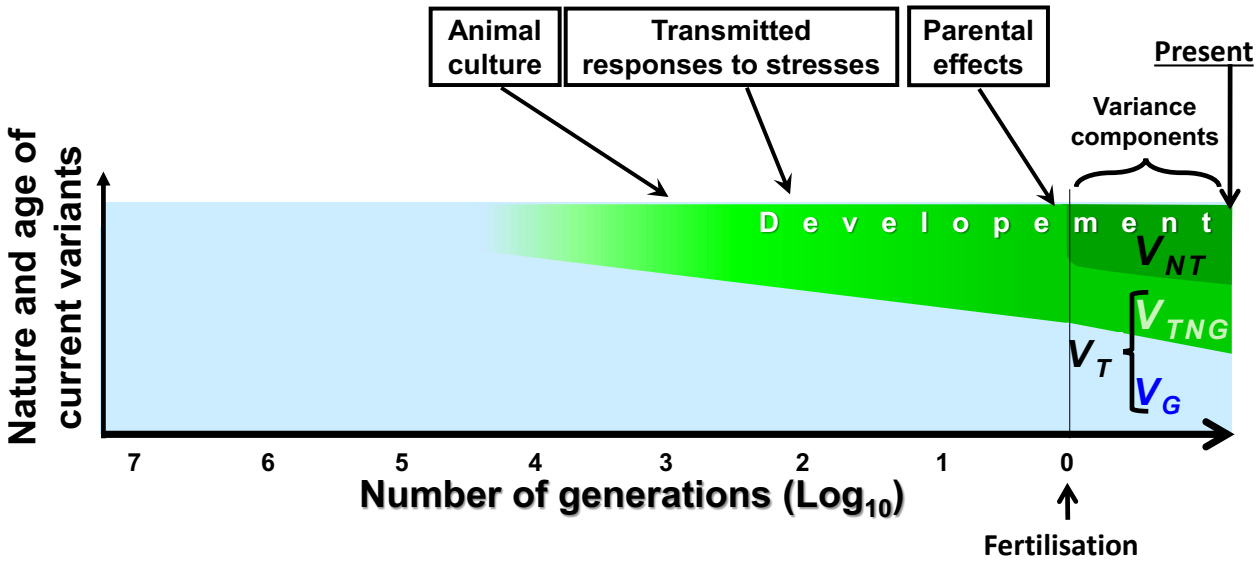
Chapter 17
The Inclusive Evolutionary Synthesis: Darwin meets Lamarck

Armed with the above information we can return to Erreur ! Source du renvoi introuvable. to answer the big question mark we left there. Our goal is to take the sixth and final step in integrating the various forms of inheritance into the Inclusive Evolutionary Synthesis that is the holy grail of this book, and which we can now make fully explicit. In Erreur ! Source du renvoi introuvable. the green area represents the environmental effects that occurred in the past and that still affect the phenotype of individuals in the current population and that together make V_E in classical variance decompositions (see Chapter 13). A central question concerns the shape of that green area. According to the Modern Synthesis, it is strongly bounded in the past by the time of fertilisation, with the minor exception of parental effects. This is why in Erreur ! Source du renvoi introuvable. the green area spilled slightly to the left of the thick boundary corresponding to the time of fertilisation. This boundary visualises the Weismann barrier, which states that traits acquired by parents cannot be passed on to their offspring.

The synthetic diagram of inclusively heritable information flows

However, we now are in a position to argue that the existence of non-genetic inheritance and its battery of sophisticated molecular mechanisms suggests that the Weismann barrier can be bypassed by various mechanisms. Clearly, a suite of traits acquired by relatively recent ancestors can be transmitted in various ways to offspring over a non-negligible number of generations.

Figure 23: Dynamics of the sources of current phenotypic variation according to the Inclusive Evolutionary Synthesis. As in Erreur ! Source du renvoi introuvable. the horizontal axis represents time in the past, and the vertical axis represents the proportion of genetic (light blue) vs. environmental (green) variants in the current population that already existed at any time in the past and has therefore been transmitted to the present. The decomposition of phenotypic variance as we developed it in Figure 17 is displayed on the right-hand side of the figure using the same terminology (V_T: transmitted variance, V_G: genetic variance, V_{TNG}: non-genetically transmitted variance, V_{NT}: non-transmitted variance). This figure makes four major changes to Erreur ! Source du renvoi introuvable. 1) The extension far into the past (to the left) of the green area representing environmental effects that occurred in relatively recent ancestors. As we have seen, this extension into the past is due for example to the roles of parental effects, the transmission of responses to environmental stresses, cultural transmission etc. 2) The fact that this green zone fades as we go back into the past due to epigenetically-facilitated mutational assimilation, 3) the virtual disappearance of the thick boundary at the time of fertilisation (Weismann barrier), and 4) the fact that development begins well before fertilisation itself[355].



Environmental effects start long before fertilisation

While it is clear that the phenotype is influenced by environmental effects that have occurred since the beginning of the individuals' lives, one must now include the fact that it is also influenced by some environmental effects experienced by ancestors. The green zone must therefore be substantially elongated in the past, well before the time of fertilisation that gave rise to the current individuals (Figure 23). In fact, this green zone must extend back at least ten thousand generations because, for example, we have seen that certain epigenetic marks or cultural processes can potentially persist over this type of time scale.

Inheritance systems interact

The further back in time we go, the more this green area fades into blue, which is the colour visualising the genetic variants of the time that still persist in the current population. We have seen, that at this time scale, epigenetically-facilitated mutational assimilation has had enough time to start engraving responses to environmental changes that have persisted from that time to the present day into the DNA sequence. Thus, although relatively slow, epigenetically-facilitated mutational assimilation has time to unfold at an evolutionary timescale.

Development begins long before fertilisation

The question then arises as to when development actually begins. The answer to this question, which may at first seem trivial, depends on what we mean by development. If we are talking about embryonic development then the answer is simple, embryonic development begins at fertilisation. This is the classical view. But, if by development we mean the construction of the phenotype resulting from the interaction between sequencic information and environmental information, then it is clear that development began long before fertilisation (i.e. throughout the green zone in Figure 23), because this interaction between the two major sources of information, genetic and environmental, began in the ancestors, who then passed on the modifications of the phenotype that they adopted in response to the environment in the form of epigenetic states (Figure 23). Admittedly, this is a rather surprising conclusion.

Darwin meets Lamarck, at last

On the basis of the framework in Figure 23, we can now summarise all the previous steps (Figure 24). In these figures, the light blue areas represent the sequencic transmission. The green areas show the past environmental effects that are still present today, with dark green showing the effects that are not transmitted (which produce the V_{NT} component of phenotypic variance V_P), and lighter green showing the part that is transmitted non-genetically (V_{TNG}). Development takes place throughout this green area.

The very long-term (left of Figure 24) is the domain of genetic transmission. This is a very reliable but irreversible mode of transmission based on the DNA sequence. This is the part of inheritance emphasised within the Modern Synthesis of Evolution. In the short to medium term of evolution (centre of Figure 24), is the area where non-genetic inheritance provides a selective advantage. This is the main addition made by the Inclusive Evolutionary Synthesis, which concerns modes of transmission that are less faithful, but which can still be transmitted over large numbers of generations while remaining reversible in the event of further environmental change. The main macromolecule involved is RNA. This domain can be called Neo-Lamarckism because it involves the transmission of certain types of responses to the environment over many generations. On the right-hand side of Figure 24 is development, which in fact extends over a much larger timescale than usually considered. It is superimposed on the green zone, and there is thus full continuity and a strong overlap between all these processes.

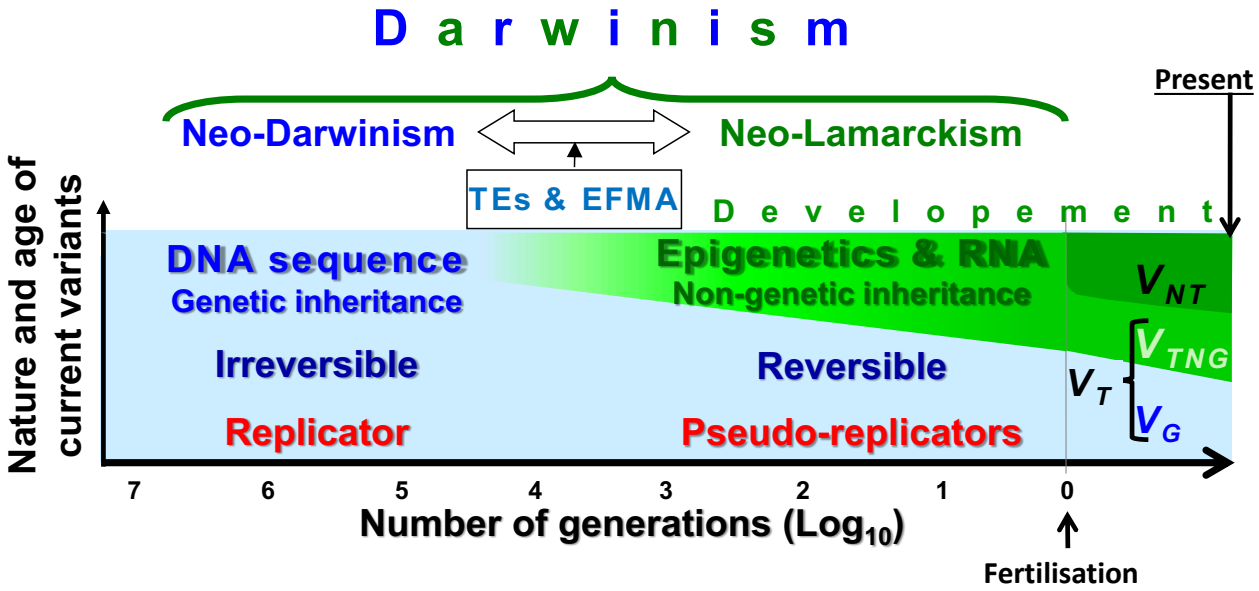
The timescale of each inheritance system is based on the existence of the other systems

Hence, the various inheritance systems should not be seen as opposed to each other, but rather as complementary to each other. Moreover, as we saw in Chapter 10, these various types of inheritance systems talk to each other in the sense that they strongly influence each other (dialogue visualised by the double white arrow between Neo-Darwinism and Neo-Lamarckism in Figure 24). Of course genetic information affects the ability to transmit non-genetic information. But the reverse is also true through epigenetically-facilitated mutational assimilation (EFMA in Figure 24), which itself results from the fact that (*i*) epigenetic marks are mutagenic and (*ii*) interact with transposable elements in affecting the levels of mutation in those parts of the genome involved in the immediate response (acclimatisation) to the concerned environmental stress (Chapter 10). This property makes it possible to explore, over a large number of generations under the same environmental conditions, the possibilities of a genetic engraving of the concerned response.

We saw in Figure 15 that each of the various inheritance systems have a specific range of transmission fidelity, each corresponding to their frequency of change over time. Although their fidelity windows probably overlap, each of the various inheritance systems occupies a different window of rate of change that makes them

suitable for transmitting adaptations to different types of environmental change. Historically, it is the specialisation of each inheritance system over one window of fidelity that has enabled other systems to specialise over another window of fidelity. In particular, it is the set of non-genetic inheritance systems that have allowed genetic inheritance to reach levels of transmission fidelity that would make it unsuitable to allow adaptation to all kinds of environmental changes occurring too frequently if the genetic system was the only ones involved. Indeed, the environment is far too dynamic for the transmission of genetic information alone —which is so faithful that it is in a sense "sanctuarised" in the DNA sequence— to be able to adapt to the incessant changes that occur in environmental conditions.

Figure 24: The Inclusive Evolutionary Synthesis, or when Darwin meets Lamarck. As in Erreur ! Source du renvoi introuvable., Erreur ! Source du renvoi introuvable., and Figure 23, the horizontal axis represents time in the past. Each increment of the horizontal axis represents the addition of a zero in the number of generations in the past. So 7 corresponds to ten million generations in the past. The vertical axis represents the proportion of genetic (light blue) vs. environmental (green) variants in the current population that already existed at any time in the past and has therefore been passed on to the present. See text. **TEs**[356]: transposable elements; **EFMA**: epigenetically-facilitated mutational assimilation[357]. Here the term genetic is understood in its current sense of sequencic. Similarly, the term Neo-Lamarckism is taken here in the very broad sense of inheritance of acquired traits[358].



A Fourier transform of phenotypic variation over generations

The multi-timescale nature of inheritance may bring to mind an analogy with Fourier transforms for breaking down a time series into its various fundamental frequencies[359]. Inheritance, and more generally phenotypic variation, with its multiple timescales ranging from a millionth of a generation to millions of generations, could be considered as functions of time analogous to sound waves.

Sound waves (and other functions of time) can be analysed by performing a Fourier transform, which allows the sound signal (or more generally a time series) to be decomposed into a series of fundamental frequencies that make it up. Therefore, if one could document the time series of phenotypic variation in a population on timescales ranging from intra-generation to a very large number of generations, one could imagine applying a Fourier transform to it. This would make it possible to show the frequency components that underlie this time series, each corresponding to one of the mechanisms of inheritance taking place on its own timescale, from intra-generational phenotypic plasticity, to the various mechanisms of non-genetic inheritance, including parental effects, ecological inheritance, epigenetic inheritance and cultural inheritance, up to sequencic inheritance that unfolds over a rhythm of up to hundreds of thousands of generations (see Figure 15).

Reversibility is a strength

Finally, all the various types of inheritance systems can be gathered under the integrative umbrella of Darwinism (Figure 24), because Darwin's vision of inheritance was much more inclusive than the one towards which the Modern Synthesis has gradually tended. In this framework it appears that, far from being a weakness, the lower fidelity of non-genetic inheritance must, on the contrary, be seen as a real strength that has allowed

the specialisation of genetic inheritance up to the very high levels of transmission fidelity that we observe, because the adaptation on shorter timescales was ensured by the lower fidelity and hence greater reversibility of non-genetic inheritance.

No need for any external action

I am sometimes told that non-genetic inheritance seems to lend credence to, or create the circumstances for, the view that evolution requires the intervention of an external force, to be called whatever you like, which would direct the whole of life according to a preconceived plan.

Insofar as the Inclusive Evolutionary Synthesis is built on the foundation of the Modern Synthesis of Evolution, I do not see what in my speech can lead to such a conclusion. The central point is that of natural selection. From the moment when (*i*) there is variation —and it is unavoidable—, (ii) that differentially affects individual fitness, and (*iii*) if this variation is transmitted, whatever the transmission mechanism involved, evolution will necessarily take place. This fact was acquired more than 160 years ago, and it is a solid and sufficient foundation. So the debate is not about that, but about the transmission mechanisms responsible for transmitted resemblance, i.e. the mechanisms of inheritance [what produces condition (*iii*) above].

A general framework for the Inclusive Evolutionary Synthesis

Figure 24 calls for further important comments, which are illustrated in Figure 25.

Figure 24 provides an integrative framework

First of all, Figure 24 *is the sixth and final step towards the Inclusive Evolutionary Synthesis*. It synthesises the previous ones by integrating all of them.

- The horizontal axis is explained by Figure 15, which is recalled on the bottom right corner of Figure 25.
- The new understanding of the sources of variation among individuals of Figure 16**B** (recalled on the middle right of Figure 25) shows off in the variance decomposition on the right side of Figure 25.
- The extension of the green zone far into the past before fertilisation incorporates the ideas of Figure 18 and Figure 19, each recalled on the top left part of Figure 25, and each connected by a large blue arrow to the extended green area of Figure 24.
- Finally, the generic mechanism of environmental action on accommodation, transmission and genetic assimilation of Figure 22 is recalled on the top right corner of Figure 25), each being linked to Figure 24 by a specific arrow: grey for accommodation that make up V_{NT}, green for adaptation that makes up V_{TNG}, and light blue for genetic assimilation that makes up V_G. Moreover, it is the third output of Figure 22 that explains why the green area eventually fades into light blue when going back in time under the influence of epigenetically-facilitated mutational assimilation (EFMA) that results from the effect of transposable elements (TEs) and the mutagenicity of epigenetic marks.

Figure 24 modernizes the Modern Synthesis

Figure 24 is built on the structure of Erreur ! Source du renvoi introuvable., that visualises the Modern Synthesis. The latter is therefore still entirely present in Figure 24. Thus, far from contradicting the Modern Synthesis, the Inclusive Evolutionary Synthesis constitutes a development that clarifies and, above all, generalises it, thus resolving some of the enigmas such as the accumulation of solid arguments in favour of non-genetic inheritance. The Inclusive Evolutionary Synthesis modernizes the Modern Synthesis[360], and in no way challenges to it.

This does not mean that this modernisation will not change the general functioning of evolution. I am even convinced of the contrary, but we will have to wait for further progress in knowledge to know whether my intuition is sound or not. However, we will return to the question of the immediate implications of the Inclusive Evolutionary Synthesis in the fourth part of this book.

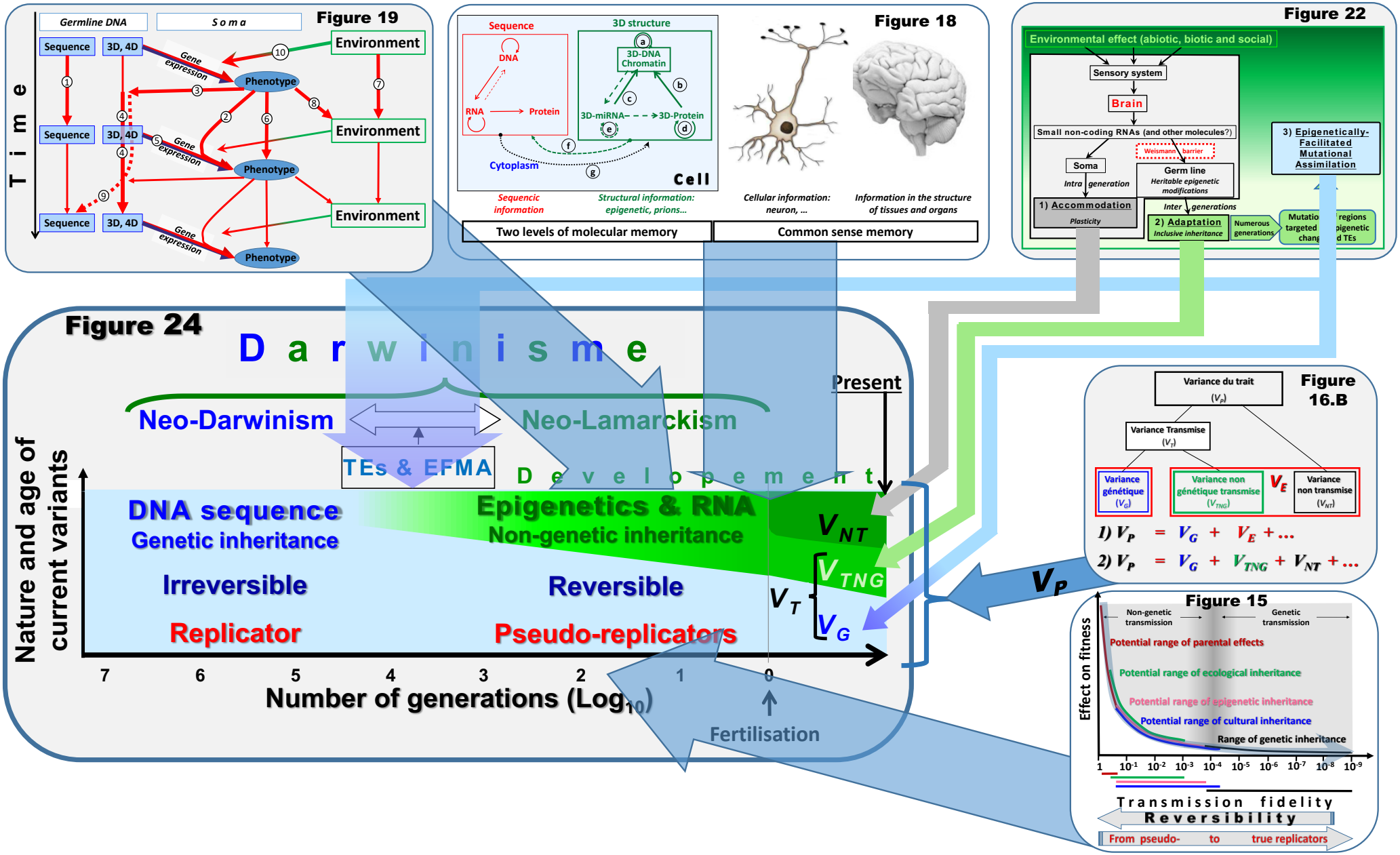
Finally, as Figure 24 shows, the Inclusive Evolutionary Synthesis returns to the broad and inclusive view of inheritance that prevailed before the discovery of DNA, which was of course the case of Darwin, who for instance included what we now call cultural transmission in inheritance.

A new definition of evolution

Finally, this view of inheritance and evolution suggest a new definition of evolution. The classical definition of evolution that can be traced back to Fisher [361] is that "*evolution is the process by which the frequencies of genetic variants in a population changes over time*".

The Inclusive Evolutionary Synthesis leads to an inclusive definition of evolution that differs from the previous one by only one word, "*evolution is the process by which the frequencies of variants in a population changes over time*"[362].

Figure 25: The Inclusive Evolutionary Synthesis. Each of the figures recalled here is the subject of one of the chapters 13 to 16. See the text for more details.



In these two definitions the word variant has exactly the same meaning and sanctions the fact that individuals within a population show phenotypic differences. The presence of the qualifier 'genetic' in the first definition illustrates the fact that for the Modern Synthesis, only the variation of a genetic, i.e. sequencic, nature matters. The simple omission of this qualifier in the inclusive definition allows for the inclusion of all types of variants, whether genetic (i.e. sequencic) or non-genetic (a term that includes all the variants discussed above, i.e. epigenetic, cultural, ecological, prion, chaperone, cytoplasmic, or microbiota variants). Since all of these variants have components that can be transmitted from parents to offspring (i.e. are inclusively heritable), these variants are all likely to evolve over time through natural selection or drift, thus participating in evolution.

Conclusion

Whereas the Modern Synthesis was the result of the convergence between Darwinians and biometricians (or if you prefer quantitative genetics), two fields that address processes occurring at supra-individual scales, the Inclusive Evolutionary Synthesis aims to integrate molecular biology, physiology, developmental biology, and neurobiology, all scientific domains that deal with infra-individual processes within and during the lifetime of an individual[363]. We will see in the last chapter that this is a real challenge because for a very long time biology has been organised globally on the basis of a separation between infra- and supra-individual approaches to life[364]. Infra-individual approaches are often linked to health research, whereas supra-individual approaches are linked to environmental research. These two scientific domains do not talk to each other very much, and often do not respect each other. They are also very often in strong competition for access to funding[365]. This is a harmful situation that must be overcome if we are to reunifying these two major fields of biology within the Inclusive Evolutionary Synthesis.

In effect, the ambition of the Inclusive Evolutionary Synthesis is therefore to unify all the major fields of biology, which requires each of them to be truly open to other fields. This is a particularly exciting objective that requires everyone to change their judgement and working habits. For example, researchers must change their reading habits by reading beyond their scientific domain to seek out information from other fields of biology. If, for example, as a specialist in evolution, I had only read evolutionary ecology journals, and there are many of them, I would not have been able to discover any of the examples developed in the second part of this book, nor those in the fourth section that I will discuss now.

Part Four

What difference does the ISE make to everyday life?

Two recurrent reactions are that all these discoveries are certainly interesting, but they do not change much in the way evolution works, and all these processes are already taken into account. This attitude is like the one we talked about earlier, which consists of clinging to the old model to avoid having to change things too much. As we have seen, this amounts to denying the concept of emergent properties, which states that the properties of the whole entity somehow escape the properties of its components. Such reactions are understandable, and they force the proponents of the emergent view to sharpen their arguments and to bring new facts showing the strength of their conception in order to make it indisputable and unavoidable. In this last section, I will not discuss whether the Inclusive Evolutionary Synthesis brings fundamental changes, because as we have seen it is still far too early to answer this question. Instead, I will illustrate how taking into account non-genetic inheritance allows us to make progress in the understanding of a series of points, of evolutionary, conservation or medical relevance to develop immediate applications for the sustainable functioning of human societies.

Chapter 18
Inclusive inheritance solves evolutionary enigmas

Why is it that in most human populations, adults can no longer digest milk, while in others adults have no digestive problems associated with milk ingestion? Why is the distribution of certain hereditary diseases in humans sometimes so surprising and counterintuitive? How can male phenotypic variation persist despite the enormous selection pressure exerted by female through their mating preferences? These are all questions to which the Inclusive Evolutionary Synthesis has already shed new light, despite the fact that it is still emerging. I am not seeking exhaustiveness here, but I want to illustrate the extent to which the new synthesis has a higher explanatory and predictive power than the Modern Synthesis.

Inclusive inheritance explains the genetic structure of some populations

Sometimes the genetic structure of populations cannot be explained by the known processes of genetic inheritance. This is particularly the case for human populations where the only way to understand how the variation of certain genes is structured within populations involves non-genetic processes such as the cultural process with its originality in terms of transmission as we have seen previously. A good example was provided by *Evelyne Heyer* of the *Musée de l'Homme* in Paris concerning the population of Saguenay-Lac-Saint-Jean in Quebec, where demographic and genetic analysis led to the demonstration that the structure of that population could not be explained without the cultural transmission of fitness components[366]. This phenomenon is far from being anecdotal, as it concerns a large number of genes, such as those involved in milk digestion in different populations throughout the world among many other genes[367].

Similar results were obtained in whales[368] and dolphins[369] where the effect of cultural inheritance is revealed by a clear lack of covariation between genetic and cultural variations showing that the latter is not simply the result of genetic variation and follows its own rules of transmission. All these data suggest that cultural inheritance is an important evolutionary process that can change the evolutionary destiny of populations and that also needs to be taken into account in both medicine and conservation biology.

Non-genetic inheritance can lead populations to new trajectories

As already stated, my intuition is that non-genetic inheritance must change a number of things in the way evolution works, leading populations towards evolutionary trajectories that could not exist if inheritance were only genetic. In comparing **Erreur ! Source du renvoi introuvable.B** and **Figure 19,** (respectively summarising the vision of inheritance according to the Modern Synthesis and the Inclusive Evolutionary Synthesis), even a non-mathematician can imagine that the equations describing the evolutionary dynamics in such contrasting worlds will differ sharply and therefore probably have contrasting properties.

a sup
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For example, theoretical approaches suggest that cultural inheritance changes many things in evolutionary dynamics because of the horizontal transmission allowing the spread of a trait within a population and changing selective pressures in the form of what some call social selection[370], a concept that includes sexual selection. For example, in the context of mate choice the social transmission of sexual preferences can quickly lead to the fixation of traits of the other sex, even if that trait is less favourable to genes than others[371].

Non-genetic inheritance as a solution to the lek paradox

Sexual selection explains why in most species females are the choosiest in mate choice[372]. The evolution of sexual preferences in females can be explained by direct benefits to females, for example in the form of nuptial gifts provided by males prior to mating, or because the secondary male sexual characteristics reveal that they do not pose a risk of parasitic infections during copulation for instance. Males may also provide indirect benefits in the form of paternal care, with females benefiting through their offspring, hence favouring females that prefer males with traits revealing their caring ability.

However, female sexual preferences also exist in species where males only transmit sperm to females and provide no direct or indirect benefit. Such reproductive systems constitute an enigma called the lek paradox[373]. In such species, the average mutation rate being much lower than the strength of sexual selection by female preferences, any variation in male fitness-related traits should quickly disappear. As a result, female sexual

preferences should also disappear in view of their costs. The paradox is that despite this, in these species, male variation is often high and preferences persist in females. Many authors tried to resolve that paradox[374]. For example, conditions could change too often (e.g. through the effect of parasites[375]), which would prevent natural populations from reaching equilibrium. However, the evidence in favour of these mechanisms is rather contradictory.

In this context, *Russell Bonduriansky* of the University of New South Wales in Australia and *Troy Day* of Queen's University in Kingston, Canada wondered whether the existence of costly preferences could be maintained through the great capacity of non-genetic inheritance to produce inclusively heritable variation. We have seen numerous examples where variation is transmitted by males. Their model makes a number of important changes to previous models addressing the lek paradox. In particular, they include the consequences of the male capacity to transmit their condition acquired during development over one or more generations, thus clearly including a fundamental property of non-genetic inheritance. Their model also does not presuppose the pre-existence of genetic variation in male quality in the population, contrary to what is assumed by almost all other models on this issue.

They found that non-genetic transmission can maintain the existence of costly female sexual preferences in the absence of any genetic variation in male fitness. Their model thus provides an explanation for the lek paradox in the absence of any genetic variation in males. We also saw in **Chapter 10 Randomness and mutation**

After discovering all these fascinating pathways of intergenerational information transfer, it is now necessary to develop an overlooked but basic property of epigenetic marks that is linked to a recurring issue in evolutionary biology, namely that of the randomness of mutations of all types. We have seen that one of the basic principles of the Modern Synthesis is that mutations are in no way directed by the environment towards improving the adaptation of organisms. Unfortunately, this principle is often simplified into saying that mutations occur at random, which does not mean the same thing. But what exactly is the case? This is what we will look at in this chapter.

Epigenetic marks are mutagenic...

The starting point that led me to think about the issue of mutation randomness was the fact that epigenetic marks, such as the presence of methyl radicals on cytosines, destabilises DNA and greatly increases the mutation rate of methyl-cytosines into thymine, another base of the DNA sequence. This, therefore, has the potential to generate point mutations whereby a cytosine is replaced by a thymine. Some articles have, for example, subheadings entitled "Methylation is mutagenic". For example, studies in humans suggest that cytosine methylation is responsible for 30-40% of point mutations in the human germline. Combining the results of several authors, cytosine methylation would increase the probability of cytosine mutating to thymine by a factor of about 20,000. This is such a considerable factor that it seems very unlikely that it is a negative collateral effect of a process selected in another context (in this case DNA methylation, which is involved in the regulation of gene expression). What then could be the function of a process that destabilises the fidelity of sequencic transmission to such an extent?

This is what we addressed in a 2019 paper. We proposed a mechanism by which such mutagenic power of DNA methylation, and more generally of epigenetic marks, might have provided a real evolutionary advantage by accelerating the sequencic engraving of the initially plastic responses to environmental conditions that prove to be very persistent. We have given this mechanism the explicit but unmemorable name of *epigenetically-facilitated mutational assimilation*.

Genetic assimilation

The idea of *genetic assimilation* (see Glossary) was proposed by *Conrad Waddington* following a series of experiments in *Drosophila* showing that following an environmental stress triggering an initially plastic response, this response tends to become heritable (and therefore non-plastic) after a certain number of generations under the effect of this stress. It was therefore as if, after a few dozen generations, characters initially developed in a plastic manner in response to a given environment became 'genetically' engraved, hence the expression 'genetic assimilation'.

Genetic or epigenetic assimilation?

However, it should be noted that in this expression the term genetic was understood in its pre-DNA sense, as 'that which is transmitted', without prejudging the mechanism responsible for this transmission. In particular, while Waddington's experiments undoubtedly demonstrated that the initially plastic trait became inclusively heritable, they did not at all show that this necessarily implied a sequencic change. In effect, there was nothing in these experiments to suggest that what he observed at the phenotypic level resulted from a change in the DNA sequence. Given that Waddington had only worked over a few dozen generations —which was already a real challenge —he in fact most likely documented an "epigenetic assimilation" because the only thing his

experiments really showed was that an initially plastic trait became inclusively inheritable within a few generations. This is equivalent to what *Mary Jane West-Eberhard* called "genetic accommodation" whereby a trait can be made heritable without necessarily involving encoding in the DNA sequence. Our paper proposed that, under certain conditions to which we will return later in this chapter, this process could go as far as sequencic engraving, *if the environmental stress persists over many, many generations*.

And the Modern Synthesis assimilated genetic assimilation

It has always puzzled me that the idea of genetic assimilation has finally been 'assimilated' by the Modern Synthesis, as this mechanism is strongly reminiscent of the much-rejected idea of inheritance of acquired traits. If you think about it, Waddington's mechanism proposes that within a few dozen generations under a given constant environmental stress the initially plastic response to stress can become heritable. In fact, what has allowed the idea of genetic assimilation to be assimilated is the relative slowness of this phenomenon. Moreover, the classical interpretation of this phenomenon is that there would pre-exist some neutral and hidden sequencic variation (usually called standing genetic variation) that would be somehow revealed by the environmental stress. Natural selection would then have the time to act over the few dozen generations of Waddington's experiments to retain only those variants that happen to be, I would like to say 'miraculously', favourable. So genetic assimilation would be just a special case of natural selection. This is how the Modern Synthesis has managed to see no major contradiction in genetic assimilation. This is also how I understood it until a few years ago.

Epigenetics as a hub towards sequencic engraving

A striking result on which we have built our reasoning is that all mechanisms of non-genetic heritability seem to involve some epigenetic change. It is as if epigenetics was the backbone or hub towards which most non-genetic inheritance processes would converge. Then, as epigenetic marks destabilize the DNA, over the course of many generations, this would generate sequencic variation *in the parts of the DNA concerned by the accommodation to the environmental change*. This would lead through natural selection acting on this newly produced variation, to sequencic engraving. In a way, epigenetics would be the conductor of the orchestra made up of all the genetic information. In effect, while it is very useful to have all the sequencic information (the recipe book), it is important to use it wisely. We shall see in **Chapter 16** that this epigenetic conductor is itself under the control of the brain.

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With *Arnaud Pocheville*, then based at the University of Sydney in Australia, we modelled this idea and were able to show that such a mechanism could accelerate the transfer of epigenetic encoding to sequencic encoding by a factor of the order of magnitude of the mutagenicity of the epigenetic marks, i.e. about 20,000 times. *This is what we called the epigenetically-facilitated mutational assimilation*.

But the story does not end there, as epigenetics interacts strongly with another major source of mutation, namely transposable elements.

... and interact with transposable elements

In parallel, we have been interested in another major phenomenon that can affect both the expression of certain genes and the appearance of mutations of all types. In fact, not only can the presence of epigenetic marks affect the stability of DNA, but epigenetic marks are themselves in strong interaction with the activity of transposable elements. Transposable elements are mobile DNA sequences discovered in maize by *Barbara McClintock* at the Cold Spring Harbor Laboratory on Long Island in the USA in the 1940s. This is one of the great genetic discoveries of the second half of the 20th century. There are a variety of transposable elements that differ, among other things, in the way they duplicate. Transposable elements exist in almost all living organisms. They seem to be able to invade the genome of an entire species through a process of colonisation from a local population, and can represent a large portion of the genome (about 15 to 22% in Drosophila, 40% of the genome in humans, and up to 90% in wheat). To give an idea of the prevalence of transposable elements, in humans, more than three million human sequences are derived from transposable elements, but only a few hundred of these have retained transposition capacity. The universality and mobility of transposable elements suggest that they play an important role in genome evolution and plasticity

The activity of transposable elements is under epigenetic control

The activity of transposable elements is strongly modulated by epigenetic processes (involving methylation, histone modifications or small RNAs) which are themselves affected by environmental factors. There are several hypotheses (not necessarily mutually exclusive) explaining the interaction between transposable elements and epigenetics. In particular, the targeting of epigenetic modifications to transposable elements could be a consequence of the *exaptation* (see Glossary) of transposable elements as platforms for chromatin modification, in which case the epigenetic regulation of transposable elements could be a consequence of genome defence and regulation. As a result, environmental stresses can trigger transposition activity, either

directly or through their effects on epigenetic marks associated with transposable elements. It can be said that in most cases the mobility of transposable elements is inhibited by epigenetic marks that block their replication. However, this targeting of epigenetic marks on transposable elements also affects, as if by ricochet, the genes close to these transposable elements —with which they become partners in a kind of "transposable-element-gene duo"—, thus affecting their expression level. Beyond their important mutational effects, by duplicating themselves in the genome, transposable elements can thus affect the general functioning of the genome, among other things by regulating and controlling the activity of genes in the neighbourhood of their insertion point. Thus transposable elements affect gene activity in three different ways.

- First, by attracting strong epigenetic marking around their insertion point, they affect the epigenetic marks, and therefore the expression, of the genes with which they are in duo. It should be noted that the epigenetic marks around transposable elements can be modified by stresses bringing back their mobility, hence modifying the expression of the genes around the new insertion point.
- On the other hand, as the sequence of many transposable elements carries regulatory elements of response to the environment, their presence will directly modulate the expression of the genes with which they are in duo according to the environmental context. They therefore play a central role in the response to environmental changes.
- Finally, by their mobility within the genome, transposable elements can generate significant sequencic changes in the genome. Their mutagenic potential is thought to increase the average point mutation rate by several tens of thousands of times.

A great generator of inclusively heritable variation

Thus, the presence of transposable elements in one area of the genome can on the one hand durably modify the expression of the surrounding genes due to the strong intervention of persistent epigenetic marks inhibiting their mobility, and on the other hand generate genetic (sequencic) variation in the whole genome as a result of their mobility. Both types of variation can affect the phenotype either negatively for individuals (e.g. they are implicated in various diseases) or positively at the population level by generating variation that is inclusively heritable and therefore open to selection. In other words, while at the individual level these changes can often have negative consequences, at the population level transposable elements generate inclusively heritable variation on which natural selection can act, thus favouring the adaptation of populations to their environment.

Interactions between epigenetics and transposable elements thus constitute a real engine for the creation of phenotypic variation (targeted to specific portions of the genome) that can be inherited either sequentially or epigenetically *in response to environmental stresses*, and are thus an important factor in evolution. Such a generator of genetic and epigenetic variation can in particular explain changes in mutability within the genome following environmental stresses. Several authors have emphasised the existence and importance of such generators of inclusively heritable variation involving the joint action of genetic and non-genetic processes in the ability of natural populations to adapt to ongoing global changes under the influence of human activities.

Epigenetically-facilitated mutational assimilation

We can now synthesize this. It appears that the effects of environmental stresses can affect the expression of specific genes involved in the response to stress and affect the activity of transposable elements, two major characteristics that each have the capacity to increase the sequencic mutation rate by tens of thousands of times, which is anything but negligible.

An information transfer pathway acting over many generations

The epigenetic changes affecting the expression of genes specifically involved in the response to an environmental stress in fact have two functions taking place on two very different time scale:

- First, these epigenetic marks, which we have seen target very precise portions of the DNA, enable the individual to adapt to the current environment by finely regulating the expression of the genes involved and leading to the phenotypic response to the environmental challenge. This response is rapidly established under the effect of environmental change. This process is known as phenotypic plasticity, the ability to modify the phenotype in response to the environment.
- Second, by being inherited, those epigenetic marks lastingly affect the mutability of the concerned genes that happen to be the genes involved in the accommodation to the specific environmental change. These epigenetic marks can also affect the activity of neighbouring transposable elements, which can further increase the mutability of the concerned regions and thus the potential generation of sequencic variation. In other words, epigenetic marking would differentially mark portions of the genome for mutation, i.e. for the generation of sequencic variation and thus for the multigenerational exploration of new genetic possibilities. Far from being a cost in terms of evolution, this may on the contrary constitute a major evolutionary benefit because the sequencic variation thus generated concerns the genes actually involved in the accommodation to the specific environmental stress, a variation then open to natural selection.

This is *epigenetically-facilitated mutational assimilation* that is more than just a special case of natural selection on initially neutral and hidden genetic variation suddenly revealed by environmental change. According to our view, genetic assimilation appears as *a genuine mechanism for manufacturing sequencic variation in the parts of the genome concerned by the accommodation to the specific environment*, variation which is then open to natural selection. This mechanism calls for several important comments.

Random mutations in environmentally targeted areas of the genome

First, with epigenetically-facilitated mutational assimilation, the fundamental axiom of the Modern Synthesis that *mutations are not influenced by the environment in an adaptive direction* remains 100% valid. However, it is the simplified phrase traditionally used to simplify this axiom "mutations are random" that appears incorrect. With epigenetically-facilitated mutational assimilation the mutations generated following a lasting environmental change are indeed not influenced in an adaptive direction by the environment (the axiom of the Modern Synthesis therefore remains valid), but the parts of the genome where the mutation rate increases are actually targeted by the environment. *This is because epigenetic changes and the activity of transposable elements are themselves targeted by the environment*. There are therefore two independent scales where randomness can be expressed, that of regional portions of the DNA, and that of the local change of sequence itself. Only the second scale is unaffected by the environment, whereas the regional scale is clearly targeted by the effects of the environment in the sense that it is precisely in the portions of the DNA concerned by the accommodation to the environmental challenge that the mutation rate changes.

A necessarily slow process…

Second, even if the magnitude of several tens of thousands of increase in mutation rate seems enormous, it does not mean that epigenetically-facilitated mutational assimilation (i.e. the sequencic engraving of the adaptation) takes place in a few generations. A rough calculation predicts that such a process must take hundreds, if not thousands, of generations to become effective. Although the calculation proposed in the last note is very crude, the important point is that we should not expect epigenetically-facilitated mutational assimilation to take place very quickly, and certainly not in only a few tens of generations. And in fact, evolutionary logic even leads us to believe that this slowness is integral to the process (see below).

… which could be involved in domestication

We were certainly not the first to think about this type of genetic assimilation where the environment can be involved in generating genetic variation in the sections of the genome involved in the response to the environment. For example, one of the earliest papers on the subject dates back to 1983 in which *Hugh Iltis*, then Professor of Botany at the University of Wisconsin, formalised a scenario for the domestication of maize from teosinte, an annual plant from Central America. This remarkable scenario integrated several previous hypotheses and involved the major and massive effect of what he called a catastrophic epigenetic sexual transmutation that occurred some seven millennia ago.

Similarly, the whole literature on transposable elements claims that the environment can generate inclusively heritable variation. Regarding the idea that the environment can generate variation in certain regions of the genome, *Eva Jablonka* and her collaborators had modelled this idea without proposing a molecular mechanism. Similarly, *Michael Skinner* also foresaw and proposed the existence of such phenomena. Furthermore, researchers working on the domestication syndrome of vertebrates proposed that the stress induced at the beginning of domestication must have caused alterations in the methylation patterns of developmental genes expressed in the neural crest (the part of the embryo that will become the central nervous system), epigenetic changes that could have been fixed in the form of genetic variants to explain recurrent behavioural resemblances in the many domesticated fish, mammals and birds.

The different systems of inheritance interact with each other

This chapter thus introduced a particularly important point, namely that the different systems of inheritance (which we will summarise in **Chapter 15**) do not operate independently of each other. On the contrary, they interact and influence each other. For example, the central idea of epigenetically-facilitated mutational assimilation is that the molecular memory represented by epigenetics states interacts over the long term with sequencic memory, in a way that can potentially considerably accelerate the genetic encoding of initially plastic responses to environmental characteristics that persisted for hundreds or thousands of generations.

Chapter 11, that the transmission of female sexual preferences can also occur in a purely cultural way through, among other things, the Fisher's runaway process, which should probably reinforce Bonduriansky and Day's conclusions. This shows that taking non-genetic inheritance into account can solve famous evolutionary puzzles.

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Different types of condition dependence

Life could be redefined as the ability to adapt to environmental conditions. In other words, depending on internal and environmental conditions, the optimal response of an individual organism may vary greatly depending on multiple trade-offs between the costs and benefits of increasing or decreasing a particular trait, a characteristic usually called condition dependence. Theory predicts that such trade-offs should move the developmental mechanisms linking trait expression to individual condition towards maximising the expression of these traits given the individual's condition. It is clearly accepted that the expression of condition-dependent traits, such as sexually selected traits, should reflect the genetic quality of individuals at many loci. However, condition is also expected to also strongly depend on environmental effects, so that the definition of condition dependence includes both genetic and environmental effects. However, because it is easier to manipulate the environment than the genetic state of a population, very few studies have attempted to compare simultaneously the effects of environment and genetic quality on trait expression. *Russell Bonduriansky* and colleagues conducted such a study in the fruit fly (*D. melanogaster*) by simultaneously manipulating mutational load and nutrient concentration in 19 family lines. This led them to the surprising conclusion that the condition dependence for traits with a strong fitness effect (such as wing length or body size) would incorporate the joint effects of genetic and environmental variation, whereas the condition dependence for traits less related to fitness (such as head length) would incorporate only environmental effects[376].

There is currently no general conclusion on the impact of non-genetic inheritance

The few cases I have quickly sketched in this chapter remain rather *ad hoc*, partly because this topic is not at the core of my expertise, but also because we currently lack a generic theory allowing us to make specific predictions about the impact of specific non-genetic inheritance system on the evolutionary functioning of populations. However, a very interesting review of the importance of these various phenomena can be found in the book by Bonduriansky and Day published in 2018[377].

In any case, it will take many years of research to better understand the global effects of non-genetic inheritance on the functioning of life in general. This is one of the major areas where the Inclusive Evolutionary Synthesis still needs to develop in order to gain its credentials. However, even if only the future will tell whether or not non-genetic inheritance changes the functioning of evolution in a profound way, it can already be said that it has started to change research in two areas concerning application to real life, namely the field of medical research and that of conservation biology, two areas that we will develop in the next two chapters.

Chapter 19
Application in medicine

If there is one domain of biology in which the question of what does the Inclusive Evolutionary Synthesis bring to the world concretely, it is that of medicine, where it is already having an impact on our daily lives. It is not without reason that medical research has played a central role in the emergence of ideas considered as at least iconoclastic if not heretical by the community of researchers in evolutionary biology. In fact, it is functional biology[378] that has provided most of the examples of non-genetic inheritance developed in the second part of this book. This is particularly the case in Chapter 9, where we have seen that many environmental stresses can be transmitted over many generations to such an extent that this form of inheritance can be said to be ubiquitous. Without the contributions of functional biology, this book would have no reason to exist and it would be impossible today to propose the Inclusive Evolutionary Synthesis as I have developed it in the third part of this book.

Thus, beyond the question of whether or not the inclusive view of inheritance profoundly changes the way evolution works, it is clear that it has immediate implications and applications in medicine that are at least partially already taken into account[379]. My aim here is not to be exhaustive, but rather to outline some illustrative examples of the need to take non-genetic inheritance into account in various medical fields.

Precision medicine

For some time now, medical science has been developing the concept of personalised medicine or precision medicine for the treatment of cancer. After all, it is well known that the same treatment applied to different people with the same type of cancer and showing no major difference in health, can be very effective in one person, but not at all in another. This is particularly the case with cancer treatment, but the same could be said for most diseases. For example, the Covid-19 pandemic affect people in very different ways, with some showing no symptoms at all and others, apparently of the same age and general condition, being violently affected and even dying. In general, any doctor will tell you that it is very useful to monitor the effectiveness of a treatment precisely so as to adapt it constantly to the specific characteristics of the subject and the disease. The question raised by this fundamental observation is to understand the origin of such variations in response to pathogens.

A first explanation for such a situation is that it may have a genetic origin, i.e. that individuals differ somewhere in their DNA sequence. This tendency is amplified by the fact that, as we have seen, there is a whole series of so-called 'genetic' diseases *for the sole reason that they have been shown to be transmitted,* i.e. the offspring of people with the disease have a higher statistical risk of developing it than the rest of the population. The classic conclusion is that these heritable diseases are necessarily due to sequencic differences. And once you start thinking in this way, the only acceptable approach is to look for the mutation(s) responsible for the disease. Too often this leads to a dead-end, because, as we have seen repeatedly, hereditary does not necessarily mean sequencic. It only means that something is transmitted from parent to offspring, without prejudging the nature of that something.

In such circumstances, it is central to make no assumptions about the nature of what is being transmitted in order to avoid rushing off in the wrong direction. In particular, it is necessary to accept the idea that one of the possible origins of variation in treatment effectiveness may not only lie in the history of individuals, an aspect too often ignored, but, as we have seen in this book, may also result from the history of their recent ancestors. Believe me, such an idea is very difficult to convey. Yet we have seen that examples of this abound.

Stopping the intergenerational spiral of obesity and diabetes

The discovery that type II diabetes, once acquired, is then passed on to offspring through the male gametes was a nasty surprise because the corollary is that the prevalence of this disease can only increase from generation to generation through a snowball effect. It was even more of a bad surprise because it eliminated the possibility of a mutation playing a role. We saw in Chapter 9 that the inheritance of this disease cannot be reduced to genetic effects, making it unnecessary to search for mutated genes in order to cure the disease and break the transmission chain. From a medical point of view, however, there is a very positive side to this discovery, because since non-genetic inheritance is reversible, it is reasonable to imagine effective therapies by targeting, for example, small RNAs in the epididymis. A genetic origin of this disease would, on the contrary, imply the use of gene therapies, which remains particularly difficult to implement. On the specific

subject of diabetes, in 1992 in a book about the illusion that sequencing could quickly lead to gene therapies, *Richard Lewontin* wrote that "*Even diabetes, which has long been known to run in families, has never been tied to genes and there is no better evidence for a genetic predisposition to it in 1992 than there was in 1952 when serious genetic studies began.*"[380].

Treating the intergenerational effects of famine

Although the case of diabetes is exemplary given the number of people affected worldwide, which reaches one billion[381], and given the mechanisms of inheritance that have been discovered for that disease (**Chapter 9**), it is far from being the only case of so-called 'genetic' diseases that prove to be transmitted by non-genetic means. Similar situations exist for various cardiovascular diseases[382], for example, or diseases resulting from a long period of fasting in humans that are then passed on for at least two generations.

One particularly well-documented case of the intergenerational effect of famines concerns what the Dutch call *Hongerwinter* (literally the winter of famine) which took place in the winter of 1944-45 during which the Germans isolated a large portion of the Netherlands causing a famine for 4.5 million Dutch people. Offspring born to mothers who experienced this famine while pregnant showed a range of metabolic abnormalities leading to a high risk of obesity, diabetes and cardiovascular disease, and showed attention difficulties, and aged prematurely[383]. These effects were furthermore found in their offspring, which corresponds to the F2[384].

These observations led to the proposal of the thrifty phenotype hypothesis, according to which intrauterine epigenetic reprogramming adapts the foetus to the environment encountered by the mother during gestation[385]. Thus, if a woman experiences food shortage during gestation, their offspring would tend to be metabolically thrifty, storing food in the form of fat reserves as soon as they have a little excess. This would lead them to become obese if they then grew up in a food-rich environment. This hypothesis has been criticised, but it has the advantage of providing an evolutionary explanation for the obesity epidemics observed in populations that have recently achieved a good level of food resources. This hypothesis also provides guidance in the search for therapies for people with such syndromes.

Understanding and treating cancer

In the study of cancer, non-genetic inheritance also provides interesting insights. Generally speaking, cancers often involve the emergence of mutations in so-called "tumour suppressor genes". In simple terms, if a mutation inactivates the function performed by these genes, then tumours can form, marking the beginning of the disease. However, these mutations are themselves preceded by aberrant epigenetic states[386], which in turn probably resulted from an accumulation of stresses that can trigger the mechanism illustrated in **Figure 22**. We saw in **Chapter 10** that epigenetic marks can in themselves both increase the local mutation rate significantly, and affect the activity of transposable elements, which by moving or duplicating in the genome can also produce numerous mutations of all types throughout the genome. Thus, mutations would not be the initial cause of cancers but rather a consequence of aberrant epigenetic patterns. Therapeutically, it is clear that if we could devise treatments to restore normal epigenetic patterns during this pre-disease phase, we would be in a position to prevent the emergence of cancer. Little is known about the duration of this pre-cancer period, but it might be quite long, leading to some accumulation over the course of a lifetime, and hence to the development of cancers later in life. It is also possible that, in view of the mechanism in **Figure 22**, some of the aberrant epigenetic states are passed on to offspring, leading to the non-genetic inheritance of the propensity to develop cancer, a disease classically known as 'genetic'.

Curing maladaptive parental behaviour

In Erreur ! Source du renvoi introuvable., we described the transmission of inappropriate parental behaviour. In such situations where parental behaviour varies systematically between family lines, considering that some of these lines have mutations that lead them to fail to adopt proper parental behaviour would imply that nothing could be done but to separate the offspring from their parents. On the other hand, the improved knowledge of the epigenetic mechanisms involved in this transmission opens up new avenues of research using the fact that epigenetic marks are reversible and can be returned to a saner state. Instead of continuing to search for hypothetical mutations potentially responsible for this behavioural disorder, the research effort should rather focus on defining therapies aimed at breaking this transmission chain by treating, for example, potentially affected young adults to modify their epigenetic marks on the promoter of the sex hormone receptors in the brain, in order to restore the normal expression of these genes. Such therapies would be highly effective in treating these individuals and breaking down the transmission chain. The rodent model we have mentioned is used for this purpose[387].

Curing various stress-related illnesses

As we saw in Chapter 9, there is a whole literature on the intergenerational transmission of the effects of many stresses at least in mammals, such as the transmission of acquired depressive states. This is important information for the treatment of this type of disease. Clearly, the idea formalised by *Sigmund Freud* that many psychological illnesses probably have their origin in the history of the individuals must be further extended to the history of the individuals *and their recent ancestors*. This is a surprising extension of the view of our psyche, to say the least, but one that we must address if our aim is to truly heal some of these often very painful afflictions.

Neuro-archaeology

Some researchers, such as *Yehezkel Ben-Ari*, have gone so far as to propose the concept of the neuro-archaeology of central nervous system diseases[388], thus asserting and formalising the importance of the history of individuals in the development of neurodegenerative diseases that are currently flourishing in the world. The Inclusive Evolutionary Synthesis suggests extending this idea beyond the history of individuals from the time of conception in order to include the history of recent ancestors, in a kind of intergenerational neuro-archaeology.

Curing autism

Autism constitutes a "textbook example" of the medical dead end in which the sequencic view of inheritance can trap generations of researchers. Autism is now recognised as a group of diseases called Autism Spectrum Disorder. It is one of the fastest growing neurodevelopmental disorders in industrialised countries and is currently the most diagnosed in Canada[389], so much so that we sometimes speak of an epidemic of this disorder, even though it is a non-communicable disease that affects four times as many boys as girls.

The mother's fault

Historically, at the beginning of the 20th century *Bruno Bettelheim* attributed the origin of this illness to the lack of affection of mothers, thus making generations of mothers guilty of their offspring's illness. This view is now discredited and it is thought that autism results from a combination of factors.

A 'genetic' disease

Then autism was found to be highly heritable[390], which led to a purely sequencic interpretation with an active search for the gene(s) involved. The disease thus became a 'genetic disease' on the sole basis that it is inherited. For example, one article stated that the statistical data on parent-offspring resemblance indicates transmission by an autosomal gene[391]. A consortium has even been formed to map autism on the genome[392].

The use of identical twins also pointed in the direction of the involvement of genes responsible for this disorder. However, the use of identical twins does not provide a convincing argument for the involvement of sequencic variation because identical twins not only received the same DNA sequences, but also received the same epigenetic information[393], prions, and other forms of cytoplasmic inheritance, parental effects, and microbiota, and most often the same environment[394]. As we saw in Erreur ! Source du renvoi introuvable., the intergenerational paths followed by most non-genetic information match the one followed by genetic information. Consequently, in all heritability estimates, non-genetic effects on resemblance are captured together with genetic effects.

In the 2000s, papers began to argue that the transmission of autism should involve many genes, with many interactions among those genes and between genes and the environment[395]. This is probably true. But such multigenic inheritance is unlikely to lead to the high heritability observed for autism. In fact, all these results point rather to a real role of one or more forms of non-genetic inheritance, which would have the added advantage of explaining the epidemic aspect of this disorder.

In fact, it is now estimated that mutations of all types explain about 50% of this disorder[396]. However, for all the reasons mentioned above, it is not unlikely that this is still an overestimate of the weight of genetic inheritance, given the difficulty of separating the sequencic from the non-sequencic components of inheritance. In any case, this result means that a minimum of 50% of the transmission remains to be explained.

A surprising role for the gut microbiota

And then there was *Ellen Bolte*. In 1998 she published a rather surprising and daring hypothesis[397]. Based on the observation that a significant percentage of autistic people had undergone strong antibiotic treatments early in life and presented recurrent and sometimes violent digestive problems[398], she assumed that the strong destabilisation of the intestinal microflora caused by antibiotics could have favoured the development of microorganisms such as *Clostridium tetani*, which is known to produce a powerful neurotoxin, and which, through its effect on the brain, could cause autism. She also relied on the fortuitous observation that a few

autistic children had shown a marked reduction in stereotyped behaviour after treatment with vancomycin, an antibiotic affecting *clostridia*. She then suggested to a doctor that he treated her own autistic son with vancomycin, and as he showed remarkable behavioural improvements during the treatment, she convinced several researchers to do a similar study on several autistic children, which they published in 2000[399]. Their hypothesis was initially about a so-called regressive form of autism that starts relatively late in a baby's development, around 18 months. Eight of the ten children involved showed strong improvements that unfortunately disappeared when the treatment was stopped.

Since then, this idea has not developed as it should have, partly because it has provoked a series of controversies, some of them rather nasty[400]. Many of the subsequent studies merely revealed the presence or over-representation, or absence, of certain microorganisms in the gut of autistic children, which was purely correlative and did not allow causality to be studied, while causality is necessary if we are to develop effective therapies.

Today, it seems established that the gut microbiota plays an important role in the development of some forms of autism, as evidenced by recent review articles and the numerous papers published on the subject[401]. In particular, a remarkable recent study involving gut microbiota transplantation showed very positive effects on both the functioning of the digestive tract and the behaviour of treated autistic offspring, improvements that persisted over the 8 weeks monitoring that followed implantation in that study[402].

A co-action of transmission processes

Today, it is accepted that a whole series of environmental factors, such as pre- or post-natal exposure to chemicals or drugs, air pollution, stress, maternal infections and dietary factors contribute to the emergence of this syndrome. But interest in the gut microbiota remains very strong, because it underlies many of the effects mentioned, and because 90% of autism cases are associated with major gastrointestinal disorders. This interest has been reinforced by the discovery of the existence of a real communication axis between the central nervous system and the digestive tract and its trillions of microorganisms. The role of this axis is becoming increasingly important for the health of multicellular organisms, suggesting that *Ellen Bolte*'s initial intuition more than two decades ago was particularly premonitory.

Thus, as should have been anticipated for a disease as complex and multifaceted as autism, it turns out that, while it is likely that genes are involved in the development and transmission of this disease, this inheritance massively involves several non-genetic factors, most notably the transmission of the gut microbiota. We saw in **Chapter 15** that the microbiota is transmitted from the mother to her baby at the time of birth, either by ingestion at the time of birth by the vaginal route, or by the colostrum, the first milk produced by the mother, which contains, among other things, many bacteria that serve to transmit the maternal microflora to the baby. In fact, the intestinal microbiota of babies born by caesarean section differs significantly from that of babies born vaginally, and it seems that regardless of the parents' condition, children born by caesarean section are more likely to develop autism spectrum disorders[403]. Similarly, premature babies have a different microbiota from other children —probably due to heavy use of antibiotics[404] to ensure their survival—, as well as a greater risk of developing autism.

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The babies' diet also seems to be involved. Breastfeeding was often absent or very short in autistic children. Later in childhood, eating habits seem to have a strong influence on the composition of the gut microbiota, and autistic children show strong food preferences for low-quality, highly processed products. We must not forget that the intestinal microbiota is strongly influenced by the food we ingest. Since eating habits are very strongly transmitted culturally, this could further reinforce the transmission of autism and explain the high heritability of this disease. Social transmission is also mentioned in a study on face-reading behaviour which showed that some parents of autistic children have a way of reading faces which is singularly homogeneous and significantly different from that of parents of non-autistic children[405].

If it turns out, as I believe is becoming increasingly plausible today, that a substantial part of the development of autism is based on the transmission of the microbiota, this would constitute a sufficiently powerful form of non-genetic inheritance to explain the high heritability of this disorder, as well as its epidemic nature. Moreover, it would provide a basis for further research, for example, by exploring further the links between method of delivery, infant feeding and autism, to test whether autistic mothers who do not breastfeed their offspring are indeed much less likely to transmit their disorder. If so, this would provide a simple method of interrupting the transmission chain of this disease that is taking such a toll in the developed world. Coupled with early microbiota transfer, we may finally be able to start treating this disease effectively.

Studying non-genetic inheritance leads to new therapies

The case of autism is exemplary because it shows how blinded we are by the purely sequencic interpretation of parent-offspring resemblance. This story shows how this can lead us in the wrong direction and delay for decades the development of effective treatments for diseases like autism, which do not need to be explained in terms of their impact on the people affected and all those around them.

Studying inheritance means investigating the causality of traits

Finally, this example, like all the examples developed in this book, shows a very important point: studying inheritance in all its complexity is tantamount to studying the causality of traits and therefore, beyond the important improvement in the understanding of the functioning of living organisms that this brings, it also opens the way to the design of new therapies for so-called "genetic" diseases.

In order to fundamentally change the way we think about inheritance, it is urgent to stop using the term "genetic disease" and to replace it with "heritable diseases", or better, "inclusively heritable diseases", which would infinitely better describe the reality of the evidence: these diseases are indeed inclusively heritable in the sense that offspring of affected individuals have a greater risk of developing the same disease as their parents. The term inclusively heritable disease would leave the mechanistic interpretation of resemblance completely open, thus avoiding locking us into a single medical approach, which would be to the benefit of the society as a whole.

Chapter 20
Potential implications for conservation biology

We are all aware of the reality of the many ecological changes taking place around us, and in particular the importance of global warming for more than a century now. It is clear that such directional changes in an important environmental parameter such as temperature have the effect of drastically changing the selection pressures on all living organisms. Over time, such directional changes greatly increase the risk that the adaptive capacities of populations to their environment will be rapidly overwhelmed, leading inexorably to extinction. This is one of the major causes of the current biodiversity crisis. How can we mitigate or even reverse the deleterious effects of all these environmental changes for which we humans are responsible? This is one of the major questions we face and will increasingly face in our daily lives. Does the Inclusive Evolutionary Synthesis have anything to tell us about how to answer these important questions for our future and the future of life in all its complexity on Earth?

Conservation biology is the branch of evolutionary biology that aims to define strategies to optimise the management of ecosystems and natural populations over the long term based on knowledge developed in biology and particularly in ecology and evolution. This scientific domain has emerged only recently, in the last five decades. Its aim is not, as is often thought, to conserve as many different species as possible, but *to conserve the diversity of ecological and evolutionary processes* that enables ecosystems and thus populations to be maintained in sustainable states in the short, medium and long term. Such an objective can only be achieved effectively if there is a thorough understanding of the eco-evolutionary mechanisms that ensure the natural functioning of ecological systems at all scales of time and space. Therefore, there is a close link between the synthetic evolutionary theory and conservation biology.

Historically, conservation biology developed mainly from population biology and population genetics on the one hand, and demography on the other. Because of their focus on the importance of genetic diversity, these disciplines have naturally insisted on the importance of conserving not only species diversity, but also —and even more fundamentally— the genetic diversity of populations, which constitutes a real reservoir of potential adaptation to environmental changes. In this context, I will address briefly in this chapter the question of what the Inclusive Evolutionary Synthesis can contribute to conservation biology.

An inclusive definition of conservation biology

In this context, we realise that, just as accounting for non-genetic inheritance led us to propose an inclusive definition of evolution at the end of Chapter 17, the existence of heritable variation based on non-sequencic memory processes calls for an inclusive definition of conservation biology. Such an inclusive conception must not only optimise the conservation of sequencic variation, but also of *all inclusively heritable variants*, whether genetic or non-genetic. The latter term encompasses, as we have seen, a plethora of very different processes, each of which making a valuable contribution to the adaptive capacity of populations to environmental change.

A provocative vision, however, could be to push the reasoning even further. Indeed, since genetic inheritance is, by virtue of its high fidelity of transmission, ill-suited to allow for immediate adaptation to ongoing change, it is unlikely to provide an adaptive response to global change on its own. This leads me to believe that the emphasis in conservation biology should perhaps not be on the conservation of sequencic diversity alone, *but perhaps we should even place more emphasis on the conservation of non-sequencic diversity*. Indeed, as we have seen in the third part of this book, it is mainly non-sequencic inheritance that is involved in the first phases of adaptation to changes of all kinds, with genetic adaptation coming only in much later after a large number of generations. In a natural system, it is only later that non-genetic inheritance can, in the long term, lead to an engraving in the DNA sequence, thanks to the effect of epigenetically-facilitated mutational assimilation including transposable elements. We might as well let living things function by their own means of inheritance as they were shaped by natural selection throughout the history of life on earth. This is most likely what would be the most effective.

Thus, focusing solely and primarily on genetic variation may be counterproductive to the natural functioning of ecological systems. On the basis of the theoretical considerations in the previous chapters, it therefore appears that taking inclusive inheritance into account should perhaps lead us *to change our focus*

from genetic variation alone to non-genetic heritable variation in populations, in order to maintain the maximum rapid evolutionary potential of natural populations.

This is a counter-intuitive and deliberately provocative thought whose practical and conceptual consequences for conservation biology and for any nature conservation policy should be explored.

Animals don't do it in cage

Non-genetic inheritance may for instance help solving the recurrent issue in conservation biology that members of captive populations of highly endangered species show very little interest in sex and reproduction[406], thus precipitating relict population into extinction vortexes. This raises the question of how can we stimulate the sexual drive of highly endangered zoo populations, a question that remains quasi unexplored and that lacks quantitative data, hence hampering the designing of methods to stimulate sexual drive in zoo populations. Some years ago we had suggested a way to solve this problem that probably originates in the fact that animals in zoos are never given the choice between various alternative partners[407]. In the best case, we bring a single male from a distant zoo and expect the female of the local zoo to readily want to copulate with that male. Obviously, females need a choice between different males to be stimulated enough to engage in sexual intercourse with the preferred male. We therefore proposed to transpose the extensive literature on mate copying, which we saw in **Chapter 11**, is a form of social learning that is strong enough to generate collective preferences for a given male, to stimulate the sex drive of zoo populations.

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The design would be based on the fact that females of various species are highly sensitive to images of males of their own species[408]. For instance, we found in the fruit fly that mate copying experiments using still images of copulating conspecifics as demonstrations triggers as efficient mate copying as live demonstrations[409]. This suggest that we can use images to stimulate females of many species to enhance their sexual drive in zoos. Hence programs could anticipate the arrival of a given male (let's call it male A), by showing the target female videos of other females choosing to copulate with male A at the expense of other males B, C, D, etc. This would be equivalent to a series of demonstrations for male A, and then we predict that the target female will be far more motivated in copulating with male A when she is presented with that male than if she had not seen it being preferred by other females prior to his arrival. This is illustrative of the lack of communication among disciplines leading to the ignorance of potential solution to urgent issues, here in the domain of conservation.

Animal culture and reintroduced populations

The area of conservation biology where this type of reasoning has begun to be used is that of populations reintroduced into environments where human activities had previously led to extinction. As early as 2004, *Hal Whitehead* of Dalhousie University in Canada and his collaborators proposed to integrate cultural processes into conservation biology[410].

A year later, *Paola Laiolo* and *José Tella* from the Department of Applied Biology at CSIC in Seville, Spain, published a study of the song of Dupont's Sirli (*Chersophilus duponti*), a steppe lark from southern Spain and northern Africa, showing that the use of song variants differed strongly according to habitat fragmentation[411]. They concluded that, given the plasticity of the use of cultural variants, the characteristics of the use of these variants could be used as simple bio-indicators of the overall health of habitat patches. This was an excellent idea. They went on to argue that conservation biology should also seek to conserve cultural variants, which are likely to disappear even more rapidly than genetic variants[412]. This loss of cultural variants may also lead to a loss of adaptive capacity of populations, some of which such as the use of tools or geographical areas during migration, affect the fitness of individuals.

Their intuition was supported more than a decade later by a large study of reintroduced populations of migratory ungulates in north-western USA[413]. Seasonal migration is a way for species breeding in highly seasonal habitats to survive the harshness of winter and the virtual disappearance of food resources in their summer habitat. That study compared the migratory behaviour of two ungulates, Canadian bighorn sheep (*Ovis canadensis*) and moose (*Alces alces*), each represented by two types of populations, those recently reintroduced to a habitat and historical populations that had always lived in their current location. They found that historical populations were highly migratory, whereas the recently introduced populations initially were not migratory, but gradually became so over several decades. This change occurred as the ungulates learned the seasonal phenology of their forage. They therefore proposed that learning and cultural transmission are the basic mechanisms for the evolution of migratory behaviour in these species, affecting their long-term survival. This observation on a large spatio-temporal scale shows the importance of integrating the cultural component into conservation decisions.

Another example is the case of the reintroduction of the American whooping crane (*Grus americana*) where captive-born individuals were taken to their wintering grounds by humans in microlights during the first migration of the very first cranes of the reintroduced population. The aim of these microlight trips was to replace the role of the experienced adults who lead the young birds on their first migration in natural

populations[414]. This was an important condition for the success of this large-scale reintroduction programme, as the cranes were breeding far north of the continent and had no chance of survival if they did not migrate south to spend the winter there. This part of the reintroduction programme of a flagship species on the verge of extinction was probably an important part of the success of this conservation action.

Other authors have gone on to support the idea that there is a need to integrate the cultural dimension into conservation biology, and have supported this idea with the various international institutions involved in the conservation of biological diversity on a global scale[415].

Conclusion

We have seen in the previous examples how it can make sense to integrate all types of inclusively heritable variants into conservation biology, in order to conserve the maximum adaptive capacity for natural populations. Conservation efforts should therefore integrate all types of inclusively heritable variants as major components of the capacity of populations to adapt to ever changing environments. Furthermore, if a choice had to be made, as I proposed in this chapter, it might be better to conserve non-genetic variants first. This is because conservation actions should first aim at optimizing the evolvabilty of natural populations on a short timescale, a goal that may be better achieved by conserving heritable non-genetic variants that are, as we have seen, better suited for rapid adaptation in an ever changing world. This is undoubtedly a provocative conclusion, but I think it would be worth exploring it if our goal is to maintain as much of the capacity of species to cope with global change.

These various examples and considerations show once again how important it is to stop equating parent-offspring resemblance with sequencic transmission, which is what we do instinctively, in particular whenever, after having shown that a trait is transmitted, we limit ourselves to sequencing only, eliminating any other possibility of transmission. The many examples in this book show how this can drive us into real dead ends, delaying the possibility of developing effective therapies or conservation solutions that can meet the objectives set by society.

In other words, we all need to appreciate that the importance of sequencic inheritance is largely overestimated and is only one of the possible ways of transmitting traits, and to keep an open mind to all other possible pathways of inheritance. Sequencing should no longer be seen as an end in itself but more as a means of eliminating one of the possible transmission routes, and if necessary moving on to analyse other routes. In many cases, it is likely that thinking upstream of action would allow us to skip the sequencic phase, for example when the inheritance shows non-Mendelian properties or when the trait seems particularly complex and condition dependent, suggesting that the inheritance of this trait is probably multifaceted.

Chapter 21
An Einsteinian revolution for evolution[416]

A conclusion that emerges from the last two chapters is that even if non-genetic inheritance were to provide only secondary adjustments to the theory, it can nonetheless drastically change the way we conceive of research approaches to support actions in various domains. Moreover, this argument can be turned around. The fact that inclusive inheritance profoundly changes the way we approach societal issues such as medicine, conservation biology or philosophy in general, could be taken as a reliable indicator of the fact that there is a good chance that inclusive inheritance will also significantly change our understanding of the way evolution works. But again, only the future will allow us to assess the magnitude of changes brought up by the inclusive vision of inheritance and evolution that I advocate in this book. In this last chapter I will discuss the ambition that should drive the implementation of the new evolutionary synthesis[417].

The new synthesis must be inclusive

The Extended Evolutionary Synthesis

Most authors calling for a new synthesis of evolution call it the “Extended Evolutionary Synthesis”. It seems to me, however, that the qualifier "extended" does not offer sufficient ambition in terms of completeness. Indeed, as many of the proponents of the extended synthesis come from functional biology and particularly molecular biology, the emphasis is mostly put on the importance of development and epigenetics. According to most of these authors, the new synthesis they call for must incorporate the fantastic memory system of epigenetics. There is no doubt that this is a necessity and that it will be a major step forward.

However, as important as this goal is, it can only be an intermediate step towards the integration of *all* the pathways of intergenerational transfer of information involved in transmitted resemblance. Returning to **Figure 19**, the integration of epigenetics alone would at best integrate arrows 2, 3, 4 and 5, but would exclude the other five arrows, 6 to 10, some of which we saw in **Chapter 15**, represent a variety of contrasting mechanisms, each with very original properties (arrows 6 and 9).

This tendency to limit oneself to the integration of epigenetics would be all the stronger since the approaches classically used to study the processes represented by arrows 6 to 10 in **Figure 19** do not necessarily involve molecular approaches (at least initially). And my experience is that there is a strong global trend in biology to consider that only molecular approaches really "advance science". All this suggests that there is a great risk that the extended synthesis will be limited to incorporating epigenetics as described in **However,** before going into the description of these many striking examples, it is necessary to take the time to introduce a fascinating and rapidly growing field of organismal biology, that of epigenetics.

A recent example illustrates the reality of this risk. I was contacted by one of the authors of an article that had just been published in *Trends in Ecology and Evolution* and who, knowing my work, wanted to inform me of the publication of that article[418]. The title of this article "*Understanding 'Non-genetic' Inheritance: Insights from Molecular-Evolutionary Crosstalk*" seemed an excellent title. However, the article used formulations that repeatedly imply that non-genetic inheritance can be reduced to what they call "*inherited gene regulation (IGR)*". Insidiously, this conveyed the message that non-genetic inheritance can be reduced to its epigenetic component alone, thus forgetting all the other dimensions of inclusive inheritance that we have discussed, namely cultural and ecological inheritance, as well as inheritance resulting from the transmission of prions, chaperone molecules, niche construction and cytoplasmic variants, or microbiota variants. I very rarely respond to articles with which I disagree as it is too likely to be perceived as a personal attack, but having been contacted by *Pim Edelaar* from the University *Pablo de Olavide* in Seville in Spain, I agreed to participate to a response as I felt the stakes were too high to remain silent[419]. The main message of our response is that this view of the new synthesis amounts to repeating the reductionist error of the Modern Synthesis, since we now clearly know that non-genetic inheritance cannot be reduced to the mere inheritance of gene regulation[420]. Interestingly, the authors then replied to our comment by saying that their objective was definitely not that, and that we were therefore in complete agreement[421]. I am sure that these authors are sincere, and have no doubt about the fact that their intention was not to reduce non-genetic inheritance to epigenetic inheritance. But if you think about it, the fact that the intention of these authors was not reductionist, shows how insidiously

this simplifying tendency can creep into all our reasoning, without us even realising it, influencing even the most open-minded people like these authors.

The Inclusive Evolutionary Synthesis

More generally, considering that only the study of infra-individual processes is relevant to biology, amounts to rejecting the fact that all four approaches to evolution summarized by Tinbergen (which we discussed in Erreur ! Source du renvoi introuvable.) are legitimate and that an understanding of evolution can only emerge from a synthesis of these four approaches. In fact, the attitude favouring proximate approaches (Tinbergen's approaches 1 and 2) is unfortunately so common among biologists that it constitutes one of the major brakes for the emergence of a more ambitious new synthesis.

Ignoring mechanisms of resemblance resulting from the properties of other molecules than the DNA, or emerging at higher levels of organisation, would boil down to denying the existence of emergent properties[422], which are properties of a given entity that are more than the sum of the properties of its components[423]. This implies that the study of the properties of an entity cannot be deduced from the sole use of the concepts and tools developed to study its components. My point is that claiming that all the properties of living organisms could be summed up in the sole properties of the DNA molecule, whose sequencic and 4D structure would allow us to fully understand the complexity of living organisms would boil down to ignoring the many important properties of living entities that emerge at levels of organisation higher than that of the DNA[424].

Furthermore, the concept of emergent property is at the heart of the major evolutionary transitions proposed by Maynard Smith and Szathmáry[425]Maynard Smith and Szathmáry (1995); Szathmàry and Maynard Smith (1995)Maynard Smith and Szathmáry (1995), Szathmàry and Maynard Smith (1995)Maynard Smith and Szathmáry (1995), Szathmàry and Maynard Smith (1995)Maynard Smith and Szathmáry (1995), Szathmàry and Maynard Smith (1995). In fact, limiting the new synthesis to the addition of the role of epigenetics in inheritance would only incorporate the first three major transitions into the new synthesis, namely (*i*) the transition from replicating molecules to populations of molecules into compartment, (*ii*) from independent replicators to chromosomes and (*iii*) from RNA as gene and enzyme to DNA plus protein (i.e. the genetic code). This would ignore the five other major transitions identified by these authors. Indeed, most of the documented mechanisms of non-genetic inheritance are consubstantial with most of the eight major transitions they identified and particularly so with the five last transitions, namely (*iv*) from pro- to eukaryotes, (*v*) from asexual clones to sexual populations, (*vi*) from protists to multicellular organisms, (*vii*) from solitary individuals to sociality, and (*viii*) from primate societies to human language.

This is why I have been calling since 2010 and especially since 2013, when I first published the expression Inclusive Evolutionary Synthesis (IES), for the new evolutionary synthesis to be Inclusive rather than just Extended. These two approaches differ only in their ambition. Having been promoted by epigeneticists, the Extended Evolutionary Synthesis runs the major risk of adding only the epigenetic dimension. While this is a wonderful and necessary step, it is not enough. Our ambition must be to integrate all the mechanisms of transmitted resemblance. It is this ambition of comprehensiveness that justifies the qualifier 'Inclusive', rather than just 'Extended', in order to incorporate the effects of all the other forms of transmission that, as we have seen, can play important roles in inheritance and evolution. In other words, we must avoid making the reductionist mistake of the Modern Synthesis by forgetting once again the fundamental concept of emergent property, which would be implicit in the assertion that all the properties of living organisms could be summed up in the properties of the DNA molecule, whose sequencic and 3D, or 4D, structure would allow us to fully understand all the complexity of living organisms.

As such, the *Extended* Evolutionary Synthesis is only one step in the ongoing emergence of a new synthesis. The "*Inclusive* Evolutionary Synthesis" ambitions to be broader as it intends to integrate all known mechanisms of transmitted resemblance into the evolutionary synthesis that we are building for the 21st century.

The challenges in establishing the new synthesis[426]

How I got into non-genetic inheritance

I remember the first time I realised that inheritance might not boil down to the transmission of the DNA sequence. It was while writing the chapter about sexual selection for the textbook on Behavioural Ecology first published in French in 2005 and then in English in 2008[427]. *Frank Cézilly* with whom I was writing that chapter suggested a section on cultural inheritance and sexual selection. I did not understand what he was talking about. For me, inheritance was genetic (in the sense of sequencic) and nothing else. I was a perfect tenant of the Modern Synthesis of Evolution, and to some extent I still am.

This instilled doubt in my head and I was so fascinated by what I discovered about animal culture, that I added two new chapters to the English version published in 2008, one about social learning[428] and one about cultural inheritance[429]. I also published a review article developing ideas on *public information* (see Glossary)

use and its potential to lead to cultural transmission, and adopting a quantitative genetics approach to define culture[430]. I later discovered the many other forms of inheritance that I developed in the second part, motivating the writing of this book highlighting the multidimensionality of inheritance.

The biggest challenge for the new synthesis

In fact, heredity (patterns of resemblance) and inheritance (mechanisms of resemblance) constitute major keystones for the building of the conceptual edifice of the new synthesis, as they are ideal concepts to connect what I call "infra-individual biology" (that Ernst Mayr called "Functional Biology"[431]) with what I call "supra-individual biology" (corresponding to Mayr's "evolutionary biology"[432]). The heredity concept can bridge these two vast domains that have been separated because they focus on very different levels of organisation of living entities, and thus use very different methodologies, tools and concepts. These two main domains of biology have been separated institutionally for too long so that now, not only do they usually ignore each other, but often despise each other.

Historically, an implicit and often overlooked basic principle of the Modern Synthesis is that we can black-box the mechanisms of resemblance[433]. Unfortunately, that implicit principle splits functional biology from evolutionary biology because adopting that principle implies that we can understand evolution without caring too much about the details of the mechanisms unfolding within an individual organism, and in particular those participating to inheritance. A consequence was that the Modern Synthesis in effect only really concerned students of supra-individual biology (i.e. evolutionary biology). However, a major message of this book is that the details of mechanisms of transmitted resemblance do matter as they affect the properties of the transmission (in particular the fidelity of the inherited information). What we used to call genetics a century ago in fact encompasses a constellation of mechanisms of incredible variety and sophistication that need to be recognised, reconciled and integrated into the new synthesis. To me, reconciling functional with evolutionary biology and bringing them to work together constitutes *The* biggest challenge for the establishment of the new unifying synthesis.

I experienced that challenge first-hand while leading a large-scale project during the last 15 years of my career. In 2006, my lab joined other labs working on plant biology within a consortium of labs. In 2010, that consortium applied to a French state call for proposal in order to transform the consortium into what they called a LabEx (laboratory of excellence), that would be funded significantly (in terms of millions of euros) for 5-year terms. *Dominique Roby*, *Jean Clobert*, *Jacques Batut* and I wrote the project, which got funded under the name TULIP (Towards a unified theory of biotic interactions: role of environmental perturbations). *Dominique Roby* and I became the head of this multi-million Euro project for the next two terms, at the end of which we obtained the renewal for a third 5-year term. TULIP's essence was to lead researchers in functional and evolutionary biology to work together, hence implicitly testing in real size the Inclusive Evolutionary Synthesis. Initially, TULIP encompassed five labs involving about 400 staff (researchers, university teachers, technicians, administrators, and PhD students). In 2019, founding labs had grown in size and had been joined by two labs leading to *circa* 700 staff. In parallel, we managed to raise funds for a new building for offices and labs of the two concerned scientific domains. Finally, at the end of our second term, TULIP fostered the emergence of a TULIP graduate school in order to train our students within the TULIP philosophy.

Overall, we were quite successful in relation to our institutions, but internally, we experienced the many difficulties of transdisciplinarity. Both the functional and evolutionary halves were excellent (a prerequisite for a successful merging). We increased the number, quality and impact factor of our articles; the number of papers having authors belonging to both disciplines increased steadily over the ten years, but remained rare representing a minute proportion of our total production.

The biggest challenge in fact lay in our mentality during our internal interactions. Many speakers never fathomed that the audience was heterogeneous, encompassing members of a constellation of disciplines. Hence, many seminars remained obscure to a significant fraction of the audience, including me who, although convinced of the necessity of integrating our approaches, and after 15 years of constant effort, regularly got lost after the first few slides of talks on plant biology. I even had the impression that there was a kind of snobbism in trying to lose a good deal of the audience. I have been confronted to such incapacity to communicate with members of other disciplines every day of the ten years of my co-leading of the TULIP project. A recurrent issue is that each discipline has a different conception of causality, a topic already tackled by Mayr[434]. This is one of the Gordian knots of transdisciplinarity. I cannot recall how many times I heard the phrase "this is only descriptive", while I regularly felt "what is their scientific question?", or "yes but you still haven't demonstrated causality, you are still stuck in some kind of correlation".

However, this long experience has had positive sides to. For instance, the initial language problems were relatively easy to circumvent. Also, the best scientific moments for me were our yearly international summer schools. We invited top scientists from all over the world to give lectures in the morning and insisted for them to be pedagogical as the audience was by definition quite heterogeneous. Although all the speakers were stars in their domain, this is the only moment in which I recurrently felt that I understood the messages of functional

biologists, even towards the end of their talks when they accelerated in tackling the current challenges of their disciplines. In these enlightening moments, I regularly thought that we had a lot to do together, but I seldom felt this after our internal seminars.

My point in describing my own experience is to show an overlooked key issue for the real emergence of the new synthesis (whatever you call it). *More than ever, we must work on how we talk about our research within the project in order to make our language accessible to a much larger audience than usually.* We should not aim to convince the audience that we are at the forefront of our discipline; this is not the point. On the contrary, we should increase the pedagogy of our talks and discussions. This is the only way to get understood and to leave all doors and all borders open.

It was clear to me, right from the beginning, that the challenge of unifying disciplines that had been separated for generations would be a multi-decadal challenge, but god, that was hard to put in motion! Again, to me the only true challenge for the real emergence of a new integrative synthesis is not in the purely scientific range but rather in the psychological capacity of members from various research areas to *listen to and respect* each other, the first step to envision integration.

My other experience of this kind is in my 10-year still ongoing collaboration with *Guillaume Isabel*, a neuro biologist of memory in fruit flies, in the study of the cultural transmission of mating preferences. It has been a highly fruitful collaboration because, right from the beginning, we have respected each other and have been willing to work hard to understand what the knowledge of the other could bring into our integrative study. Guillaume and I regularly state, in a simple language, the principle of this or that technology, or the rationale of that or this question etc. and this is very useful.

To sum up, I think that the shift from the Modern Synthesis to the new synthesis is a major step for our understanding of life, but slowness is not stagnation. Any exponential growth starts very slow, and nonetheless, after a while it looks more like an explosion. Thus, early slowness in the building of a new synthesis should not refrain us from acting in that direction.

A parallel with the conceptual revolution of relativity

This ongoing evolution in biology from the Modern Synthesis to the Inclusive Evolutionary Synthesis via the intermediate stage of the Extended Synthesis is reminiscent of the history of the revolution that took place at the beginning of the 20th century with the transition from Newtonian physics to relativity, first special and then generalized. Although such a parallel may seem grandiloquent, and although it is up to historians to decide whether the ongoing conceptual paradigm shift towards a new synthesis of evolution constitutes as fundamental an advance for biology as the introduction of relativity was for astrophysics, the parallel is intriguing enough to deserve to be made here.

This parallel makes two important points. First, the fact is that today no one would use special relativity, which in fact was an imperfect temporary step towards generalised relativity, which alone is now recognised as effective. Second, as we have seen in the case of the transition from the Modern Synthesis of Evolution to the Inclusive Evolutionary Synthesis, in the case of the transition from Newtonian physics to relativity, the latter did not invalidate Newtonian physics but generalized it. So, there is an interesting parallel between these two paradigm shifts. Hence the title of this last chapter[435].

Philosophical implications

Did you say paradigm shift?

Philosophically, to answer the question of whether the new evolutionary synthesis currently emerging is likely to profoundly change our conception of how evolution works is to ask whether this change corresponds to a real shift in scientific paradigm. This question, which is in fact at the heart of many debates on the new synthesis, is reminiscent of the work of *Thomas Samuel Kuhn*, who dealt with this very question in a general way. For Kuhn, and I share his point of view, the history of scientific ideas is a dynamic history made up of a series of stages, which he describes as 'normal science', separated by phases of rather abrupt transition, which he describes as 'extraordinary science', during which real paradigm shifts take place and knowledge truly advances. According to Kuhn, the periods of normal science simply validates and revalidates over and over again in different contexts the last emerged scientific paradigm. They are therefore uncreative and unimaginative. Whereas the short periods of extraordinary science marking the transition from one paradigm to another are very rich in imagination and conceptual advances that really mark the progress of science.

This was undoubtedly the case at the beginning of the 20th century for physics with relativity, which profoundly changed our conception of the universe. Before that date, it was the Newtonian revolution that constituted such a paradigm shift. Further back in time, we can think of the revolutions introduced by *Copernicus*, then *Giordano Bruno* and then *Galileo* in the 16th century. I think it was also the case in the middle of the 20th century with the discovery of the structure of DNA and the genetic code, which marked an absolutely remarkable advance in our understanding of life. We can clearly say that there was a before and an after, and

this is why even today, more than seventy years later, we must integrate the fact that there are two concepts of the gene, the pre-DNA concept and the post-DNA concept.

I have already expressed the fact that I do not think that the question of whether the emergence of the new synthesis constitutes a paradigm shift is a central question at this stage and that it will be the role of future historians and philosophers of science to decide this, but what can be said right now is that they will have to place their reflections in relation to Kuhn's work.

I see a difference, however, between the examples of paradigm shifts I cited above and the emerging new synthesis. I purposely did not cite the case of the Modern Synthesis of Evolution as an example of a paradigm shift, but it is one nonetheless. My reason was that this paradigm shift, however influential, was in fact based on the convergence and synergy of two disciplines. Hence, the shift did not result from the work of one or a few individuals. This may be because biology has reached such a level of complexity that one person alone cannot move its boundaries. However, the fact that this change in the view of biology is not the work of one person does not mean that it does not constitute a paradigm shift in the sense of Kuhn. But it is important to emphasise here that the emerging new synthesis will only emerge if the two major fields of biology, infra-individual (and therefore strongly molecular) biology and supra-individual biology (studying interactions among individuals and beyond[436]), are able to come together and respect each other. As I said above, this is the real challenge, indeed.

A recurring reaction

Still in the field of philosophy, I often get people at the end of my talks who tell me that this new view of inheritance scares them, because it implies that everything we do could affect the lives of our descendants, which makes us infinitely more responsible towards them. I'm delighted by this kind of comment because it shows that I have succeeded in getting the main message across. This kind of reflection also shows that the audience has perceived all the philosophical and moral consequences that inclusive inheritance implies in terms of how we survive through our descendants, or about the deep nature of humanity, and in terms of inheritance and what we mean by atavism. This truly changes the very nature of life, but I don't think it should be frightening. On the contrary, it should reassure us, at least initially.

An immediate positive reaction

It seems to me that what should have frightened us was rather the Modern Synthesis' view, for according to it, inheritance is cold and blind and almost entirely beyond our control and choice. Inclusive inheritance is much more condition dependent, which should be seen as rather reassuring. There is even real poetry in knowing that beyond the rigid and unalterable transmission of the DNA sequence alone, we also pass on a great deal more that results from our own experience early in life alongside that of our close and less close ancestors. It is quite moving to see that we are even more than we thought previously the fruit of a story with all its anecdotes and multiple dimensions.

It should be noted, however, that except for cultural transmission, we only transmit responses to events that occur early in life, before reproduction or before the birth of offspring, i.e. essentially during our development. This shows the importance of early in life effects; not only can they shape the phenotype, but they can also affect the phenotype of descendants over generations.

Here again, cultural transmission is an exception in that a significant part of it takes place after birth during the period of dependence on the parents, a period which in the human species lasts more than two decade. This opens up a wide range of possibilities. Thus, even innovations (or learning) that occur in parents after the birth of their offspring, or even in old age, can affect the phenotype of their offspring. This is another peculiarity of cultural transmission which implies that the evolutionary effects of cultural transmission must be original in its effects on the course of evolution, and have yet to be explored.

Another quasi-philosophical implication is how a plethora of eminently selfish and very different replicating entities contributes to the emergence of a multifaceted process of heredity that allows each generation to adapt to the prevailing environmental conditions, and perhaps pass on the relevant adaptations, with the effect of shaping the offspring to those conditions that are likely to persist beyond the parents' lifetime. Life is therefore infinitely more complex than we thought, and this complexity emerges from the diversity of interactions between the sources of variation, all in interaction with the multiple components of the environment.

Thinking over the long-term

However, in a second step, one cannot help but reflect that the remarks of my listeners are about the risks of passing on the effects of our various mistakes and addictions to our descendants. There is, however, an unexpected facet to the inclusive vision of life that I have defended in this book, that of the consequences in the field of criminology and justice. These are two fields that must in the long run incorporate, in one way or another, the importance of the non-genetic transmission of various reprehensible behaviours, either by cultural

or epigenetic means, as we have seen in Erreur ! Source du renvoi introuvable., for example. This therefore constitutes a vast and as yet unexplored field of application of the inclusive vision of life with immediate repercussions for the functioning of our societies.

But it seems to me that all these aspects are in fact only a very small part of the responsibility that one generation has towards future generations. The problem is much broader than passing on the effect of our bad habits to our own offspring. As we have seen, inclusive inheritance goes far beyond the classical meaning of heredity, and the example of niche construction (**Chapter 15**) shows that we also pass on all the perennial changes in the environment that we have made voluntarily or involuntarily during our lives.

When these changes occur at a sufficiently slow pace and on a sufficiently small scale that the natural functioning of ecosystems has time to compensate for and erase these changes as they occur, this does not pose a real problem in the long term because the ecological functioning wipes the slate clean from every generation. But for the last half dozen generations or so, the combination of various factors such as the relentless development of technology and the economy associated with the exploitation of natural resources, all amplified by an ever faster growing human population (an aspect of the problem that is all too often ignored at the societal and political levels), has meant that the amplitude of these changes is far greater than the corrective power provided by the Earth's natural ecological functioning. This is a facet of inheritance that it would be particularly irresponsible to deny, as it lies at the heart of the issue of global change, which constitutes one of the major challenges facing humanity today and in the decades and centuries to come. As a result, we are living on credit and collectively amassing a huge ecological debt, as the consequences of our activities accumulate in the environment to the point of depleting some major resources, as well as profoundly changing the climate and oceans across the surface of planet Earth.

For more information

Glossary

Accommodation: Refers to non-transmitted and often beneficial responses of organisms in reaction to the state of the environment. Accommodation is the result of plastic changes during an individual's lifetime.

Adaptation: Classically, adaptation refers to characteristics of organisms that have been established through a history of mutation and selection. Here I use an extended view of this concept including all traits resulting from a history of selection on information transmitted regardless of the nature of this information (whether genetic on non-genetic).

Chromatin: The DNA in the chromosomes is flanked by many types of proteins that together with the DNA form chromatin. The origin of the term comes from the fact that this conglomerate of molecules is very easily stained in optical histology. This chromatin can take on two distinct histological aspects, euchromatin and heterochromatin, which we now know correspond to two different functional states. The euchromatin parts of the genome are open to be expressed, whereas the heterochromatin parts are silenced.

Chromosome: The DNA molecule in the nucleus of eukaryotic cells is cut into several separate pieces. Each piece is associated with a series of proteins that form a kind of packaging that forms the chromatin, which is large enough to be visible under the light microscope. This collection of a DNA double strand plus its suite of proteins is called a chromosome. Each chromosome exists in duplicate in all cells of diploid organisms, one from the mother, one from the father.

Conformity: The tendency of individuals in a group to adopt disproportionately the most common behaviour in the group[437].

Cytoplasm: The bodies of all visible organisms are made up of cells. A human being is made up of about 200 different types of cells for a total of 35 to 100 trillion cells, of which the nervous system alone has about 100 billion. Each of these cells is bounded by a cell membrane made up mainly of phospholipids. Typically a cell has two compartments, the cytoplasm and the nucleus, itself surrounded by a double phospholipid membrane. The nucleus contains the complete double set of chromosomes (23 pairs for humans) and thus most of the DNA of a cell. The cytoplasm contains numerous organelles, often consisting mainly of membranes. Some of these organelles (chloroplasts and mitochondria) also contain some DNA for their own function.

***De facto* finalism**: Reproduction is the ultimate finality of all biological entities, so that there is a *de facto* finality in biology, which is that it is the lineages whose properties lead them to have the highest number of descendants (i.e. having the highest fitness) that are selected for and that survive over the course of generations. See the entry about teleology.

Diploid: Organisms with two sets of chromosomes, one from the mother and one from the father, are called diploid. This is the case for the majority of living organisms that we see. Organisms with only one set of chromosomes are called haploid. This is the case, for example, with mosses, ferns and algae. All sexually reproducing organisms have a life cycle comprising a haploid and a diploid phase. Organisms differ only in the relative length of these two phases.

Drift: Drift constitutes the second main mechanism of evolution alongside with natural selection with which it shares two of the three conditions. As for natural election, evolution by drift occurs as soon as there is variation within a population and that variation is inclusively heritable. The only difference is that in drift, the selection is independent from fitness and just results from chance. Drift is mostly influential in small populations and this is why many models of population genetics assume that the modelled population is infinite in size as a way to eliminate any potential effect of drift (i.e. of randomness).

Environment: In this book, this term means everything beyond the limit of the individual. However, it should be noted that Fisher's Fundamental Theorem introduces a very original definition of the environment of a given gene that adds to the above definition all the genes in the genome and the population gene pool[438].

Epigenetics: Today, includes all changes in gene expression that are not due to variation in the nucleotide sequence of DNA and that are either transmitted during mitosis or inclusively heritable between generations of organisms[439].

Exaptation: A special form of adaptation in which a trait, feature, or structure of an organism or taxonomic group takes on a function that differs from its original function that had been derived by evolution. The classical example is that of feathers that probably first evolved in dinosaurs in the context of sexual selection,

then was exapted a first time to fulfil a thermal function and then was exapted a second time to fulfil the flight function that we see in today birds. Interestingly the thermal and sexual signal functions are still present in birds.

Evolution (classic): The process by which the frequencies of *genetic variants* in a population changes over time.

Evolution (Inclusive meaning): The process by which the frequencies of *variants* in a population changes over time.

Evolutionary biology: Term taken in the sense of Mayr (1961) to cover that part of biology which is concerned with supra-individual processes such as behavioural ecology, dispersal, all inter-generational processes, demography, population genetics, ecosystems, ecology in general and evolution.

Functional biology: Term taken in the sense of Mayr (1961) to cover that part of biology which is concerned with infra-individual processes such as molecular genetics and epigenetics, cell biology, development, cognition, neuroscience, physiology and medical sciences.

Finalism: See *de facto* finalism.

Fitness: Phenotypic fitness is the ability to have mature offspring relative to other individuals in the same population at the same time. Genetic fitness is the capacity of genetic variants to change in frequency within a population across generations. Individual fitness therefore quantifies the tendency of a type of individual to increase or decrease in proportion within a population over generations.

Genes: There are many definitions of this term. They can however, be grouped into two vast profoundly different categories[440]. The first meaning is purely statistical, a gene being anything that is transmitted leading to parent-offspring resemblance. This concept was dominant before the discovery of DNA (before 1950). After that date the term took on a second, purely molecular meaning: a gene is the information encoded in a portion of the nucleotide sequence of DNA and that encodes the amino acid sequence of a protein. This dominant view corresponds to what I call sequencic. Within this second understanding of a gene, Williams and Dawkins define a gene as any part of a chromosome that is not broken up by recombination and is therefore passed on intact across generations[441]. The conceptual duality of a gene maintains pervasive ambiguity in scientific debate, as both understandings are often unconsciously used in the same paragraph, or even the same sentence. Although this second meaning is strongly reductionist, in this book all words with 'gene' as a root refer to the molecular definition, i.e. sequencic, as this view has the merit of being both very precise and the most largely accepted concept among scientists and laymen. This semantic issue is at the very heart of the current debate on genetic and non-genetic inheritance.

Genetic assimilation: A situation where an initially plastic response to an environmental stress tends to become heritable (and therefore non-plastic) after a number of generations under that stress[442]. See Chapter 10.

Genocentrism (or gene-centric): The dominant view that heredity can be reduced to the sole transmission of the DNA sequence. Equivalent term: Sequencic.

Genotype: All (or only part depending on the context) of the genetic (sequencic) information of an individual.

Heredity (classic meaning): The classic definition is that heredity concerns patterns of parent-offspring resemblance. This term often has two meanings. 1) The fact that offspring resemble their parents. This understanding therefore focuses on the patterns of parent-offspring resemblance. 2) The mechanisms that produce this resemblance and that rely on the transmission of a wide variety of information from parents to their offspring. To avoid confusion between these two meanings, in this book I use the term 'inheritance' for this second understanding. Another interesting definition, which I cite here for the sake of completeness and as food for thought, is that "*heredity is the recurrence of developmental process*"[443].

Heredity (inclusive meaning): The classic definition above only encompasses resemblance resulting from vertical transmission, i.e. from parent to offspring and hence among relatives. But, non-vertical transmission also exists, in genes (though very rarely in eukaryotes but more commonly in prokaryotes), and much more commonly in cultural inheritance, hence fostering resemblance among non-relative members of the same population (i.e. a group of interacting individuals). A more general definition of heredity could thus be "*patterns of resemblance that result from the transmission of information among individuals*" or "*transmitted resemblance*". The term information here encompasses information with a well-defined avatar, (*i*) such as in genetic (DNA sequence), (*ii*) epigenetic information (epigenetic marks), or (*iii*) in the form of a stable molecule shape and function (prions and chaperon). It also includes information with avatars that are less easy to define, such as (*iv*) the transmission of cellular states beyond epigenetic states, or (*v*) that of the environmental state, or (*vi*) the transmission of microbiota. Finally, it also includes (*vii*) information transmitted among individuals with no real avatar as through social learning and cultural inheritance. *The important point is that such transmission should lead to resemblance that is stable intergenerationally.*

Heritability: Initially, the term was used to describe the statistical level of parent-offspring resemblance, regardless of the mechanism responsible for this resemblance. The idea was to capture that part of the variation within populations on which selection (natural or artificial) can act and allow the trait to evolve. However, the discovery of DNA and the genetic code led to a reduced view of transmission and heritability. As a result, its meaning has changed to refer only to the part of parent-offspring resemblance that is *due to sequencic variation*. Since today it is this second meaning that largely predominates, I have proposed the term "inclusive heritability" to return to the original meaning of this concept[444].

Inclusive Heritability: It is the "heredity of differences", i.e. the part of variation that is transmitted, regardless of the mechanism (be it genetic or non-genetic) responsible for this resemblance. Corresponds to the initial meaning of the concept of heritability.

Information: Any factor that can affect the characteristics of an organism [i.e. the phenotype of an individual] in a way that can affect its fitness[445].

Inheritance: See heredity.

Metapopulation: A spatially structured population made up of sub-populations that function more or less autonomously but remain connected to each other through dispersal among sub-populations.

Nucleotide: The basic building block of the DNA molecule. There are four types, Adenine, Cytosine, Thymine, and Guanine (called A, C, T and G).

Phenotype: All (or only part depending on the context) the characteristics of an individual, whether morphological, physiological or behavioural in nature.

Pseudo replicator: Replicating entities with a lower level of replication fidelity than true replicators, and which therefore persist over shorter periods of time ranging from a few generations to tens or even hundreds of generations. Pseudo-replicators therefore participate in inheritance but on shorter time scales. The most striking examples are the pseudo-replicators generated by the transmission of epigenetic and cultural states.

Public information: Information that is accessible to any individual.

Replicating entity: any entity capable of replicating itself. Includes true replicators and pseudo-replicators (see corresponding entries).

Replicator or True replicator: Entities that can self-replicate with very high fidelity and can therefore remain unchanged indefinitely. The typical example is the sequencic replicator.

Selfish: Many of the critics of the use of this term in evolutionary biology in fact conflate its two very different acceptations. Intentional (or teleological) selfishness encompasses situations in which an individual *purposely* acts in order to favour its own interest at the expense of others. This first meaning is usually applied to humans. Contrastingly, factual selfishness encompasses cases where the intrinsic properties of biological entities unavoidably leads to some of them to be favoured at the expense of others. Under this second meaning, selfishness is not intentional; it is the mere by-product of natural selection blindly favouring entities whose intrinsic properties lead them to reproduce the most.

Sequencic: This term was inadvertently coined by *Hervé Philippe* during an informal discussion to describe genocentrism, i.e. the reduction of heredity to the sole transmission of the nucleotide sequence of DNA. I have appropriated it with his permission because it describes perfectly the dominant view of Neo-Darwinism.

Simultaneous exposure: Refers to cases where parent-offspring resemblance results from the fact that the parents and offspring have been simultaneously exposed to the same environmental stress. For offspring, the exposure may have taken place while it was still as a gamete or an embryo. In this case one cannot really speak of heredity (see Table 1).

Social information: Any information extracted from interaction with other individuals or from the observation of the behaviour and performance of other individuals.

Social learning: According to two Israeli colleagues, there are at least 35 different definitions of social learning[446]. In order not to be trapped in a purely semantic discussion, by social learning I mean any situation where the fact that an individual organism (called an observer) has been able to observe others (called demonstrators) interacting with each other or with their environment affects the observer's subsequent behaviour and decisions. So, I use the term in a very broad sense. Whether this involves simple copying, or imitation, teaching or even actual learning is secondary to my use of the term in this book. What is important is that some form of information passes from demonstrators to observers.

Species: A population or set of populations whose individuals can actually or potentially reproduce with each other and produce viable and fertile offspring, under natural conditions[447].

Teleology: The idea that natural processes are directed toward an *a priori* end or shaped towards an ultimate purpose, an idea that is rooted in Paley's "natural theology"[448]. Sometime wrongly called finalism for short.

Teleology is not necessary to explain evolution, as the properties of living entities are sufficient to generate evolution in the absence of any a priori goal. A teleological interpretation results from the fact that observing *a posteriori* the evolution of species gives the impression that the evolution followed a purpose, but this is pure illusion resulting from the recapitulation *a posteriori*. In fact the evolutionary history of species followed a specific path, but this path was not fixed *a priori*, but simply resulted from the joint effects of randomness (drift) and fitness (natural selection). See the entry about *de facto* finality.

Trajectory (population's or evolutionary trajectory): the way the state of a population changes over time. This concept thus refers to the whole history of the population's states. States could concern the genetic structure, or the proportion of bold versus shy individuals within a population, or the population's proportion of dispersers vs resident, or that of bright vs dull individuals, males vs females, tall vs short individuals, fit vs unfit individuals, fat vs lean individuals (in short any phenotypic trait). The study of evolution aims at exploring the mechanisms that produce these changes in time and space. For instance, certain human populations have genetic structures that cannot be explained by DNA sequence transmission alone. The only way to explain the observed genetic structure (i.e. genetic state) is to include a cultural process in the system (i.e. cultural inheritance of some traits)[449]. Some trajectories might reach some steady state for some time meaning that the population has reached a kind of equilibrium, but, in nature, conditions might change too often for such equilibria to be reached effectively.

Acknowledgements

I don't know if you're like me, but when I get to the end of a book that I don't want to leave, I rush to the acknowledgements in the vain hope of continuing to stay in the atmosphere that I enjoyed. However, most of the time I get frustrated because the acknowledgements sections are rarely more than a Prévert-style inventory of people who helped in one way or another to imagine, write and edit the book. For those of you who would like to stay in the atmosphere of the functioning of the living world, I would like to offer you some more informative acknowledgements, while still fulfilling the important function of thanking people, plus other functions.

Just as when we try to retrace the steps that led to the emergence of an idea that has influenced the current scientific vision on a given topic, when we look back on our own life, we quickly realise that a very large number of people have influenced our development, at each of the stages that have built us. In this context it is certain that we forget important people.

Every individual is built up by a series of teachers, among others. I was lucky enough to have many remarkable ones whose vocation was to accompany young people in their intellectual development. None of them know it, but I do remember them. I will mention only one of them, *Jean Fiasson*[450], my biology teacher in the second year of the agricultural preparatory course at the *Lycée du Parc* in Lyon during the year 1972-1973. Apart from the fact that his lectures were fantastic, he was the first to give me the self-confidence I needed to become a scientist. Later, I was influenced by *François Bourlière*[451]. When we last met in 1992, when he was over 80 years old, he was still younger than many of us and had a real vision of where the evolutionary sciences should go. His passing was a real loss. *Robert Barbault*[452] was another such person. He went looking for me to continue building a real lab of ecology and evolution in Paris. It was he who forced me to teach, with all the virtuous consequences that this had on my scientific development. I still haven't recovered from his death far too soon.

There are also all the names of my colleagues mentioned in the book. *Jean Clobert* is undoubtedly one of those who influenced me the most. We have been accomplices for more than 30 years, which were the best years of my professional life. Many of the ideas I put forward here were influenced by him. *Richard H. Wagner* who has so often helped me to formulate my intuitions into real ideas, was kind enough to comment on parts of this book. I would like to thank *Armelle Barelli*, Regional Delegate of the CNRS, for her masterly support from 2005 to 2013 following my arrival in Toulouse. I learned a lot from her in the field of scientific leadership. Although a physicist by training, she understood the interest of the ideas in this book and accompanied me in my actions to organise science in the Toulouse region.

I would like to thank the CNRS, this beautiful institution which, by financing my salary from 1982 to 2018, has been the main investor in my research. For a long time, the prime objective of the CNRS was to increase knowledge, the administration accompanying the achievement of that primary goal, which gave it a remarkable flexibility. It is highly desirable that the current tendency to forget the spirit of the rule in order to think only about compliance with the rule (which amounts to giving more and more weight to the administration) be quickly reversed to enable the CNRS to maintain its international momentum. I want to dot the i's and cross the t's, what matters is not so much the letter as the spirit of the rule. Everywhere, this fundamental principle has become completely obsolete, the priority being given to the sole respect of the rules, forgetting that the role of the administration is not to direct, but to accompany the realisation of the institutions' objective. Today, the primary objective of the actors in an institution seems to be to satisfy administrative requirements, often asking them to do the job for which the administration was set up. This is a suicidal strategy.

A example of this phenomenon was revealed after the Covid-19 health crisis. The heads of the University Hospitals Centres all asked after the first wave to be left to work in their own way. During the crisis they worked as they should always work. They were particularly effective, we know, but this could only be achieved by freeing themselves from the sterilising rules. In other words, without these rules they can function very effectively, the conclusion being that rules and red tape are very expensive and hamper the very functioning of institutions. This illustrates a global phenomenon to which we would be better off reflecting on collectively. In fact, it would be enough to stop assuming that all citizens are cheaters to make significant savings. We replace the current principle of suspicion (due to the existence of a tiny fraction of cheaters) by a principle of trust, coupled of course with random, regular and severe controls. This simple change in the basic principle would easily lead to a halving of the cost of institution functioning and make the action of the staff in charge

of achieving the objective of the institutions infinitely more effective, i.e. treating people in hospitals, increasing knowledge in research institutions.

Another institution that helped me a lot is INRA (now INRAE) in the development of the LabEx TULIP, whose approach is in fact very similar to the one I adopt in this book: to study living organisms in an interdisciplinary manner by rejecting the compartmentalisations in independent scientific domains that prevents the integration of the knowledge obtained in the various sub-disciplines of biology. INRA has been a very intelligent partner to *Dominique Roby* and me in this approach. I would like to thank Dominique Roby, with whom we ran TULIP for 10 exciting years. What a fantastic adventure, and what can cooperation do!

One of the beauties of being a researcher is that you work with other researchers and students. Of course, you have to know how to detect those with whom you can make progress, but once you have found them, it is a real pleasure. I would like to mention the dream team of the cultural drosophila model, including *Guillaume Isabel* and *Sabine Nöbel*, temporarily joined by *Arnaud Pocheville*. The whole 'Cultural Flies' group has been a real boost. What a pleasure and an honour to have them as close colleagues! *Adeline Loyau* was at the origin of the invention of the hexagon and its development by *Anne-Cécile Dagaeff*. *Simon Blanchet* who, during his post-doc with me, left his beloved fish for a while to work on drosophila. Without *Sabine Nöbel*, the whole 'Cultural Flies' project and its crew of students would never have taken off. She was and still is a real motivator. *Arnaud Sentis* who, during his post-doc, worked with us on the aphid project on epigenetic inheritance. *Benoit Pujol* with whom we have had so many research interests in common although he works on plants and I on animals. *Lounès Chikhi* for his often concrete support. And all the colleagues of my laboratory in Toulouse. Thanks to all of you.

Students do not realise how important a role they play in stimulating and preventing things from going in circles. They really influence research and this is a real asset. Thanks to them for that and for the pleasure of working with them. The price to pay is that it is very sad to see them leave for new horizons when they graduate, but this sadness is the proof of success. I will miss working with students the most when I am no longer able to do science. They are remarkable people. It was not always easy, but I hope it was constructive for most of them as it has been for me. I won't risk naming them here because it would be very long and I would risk forgetting some of them, which I want to avoid at all costs. They know it very well, and if they have forgotten, that's fine.

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The genesis of all these ideas has been influenced by many, many people, and it is impossible to list them all here. I hope that people who recognise themselves as having participated in some of the ideas developed in this book and who do not see their names here will forgive me this omission. I can mention, in alphabetical order (and citing only those whom I did not quote above), *Jean François Arnal*, *Pierrick Blanchard*, *Thierry Boulinier*, *Louis Casteilla*, *Frances A. Champagne*, *Isabelle Coolen*, *Jean Deutsch*, *Niels Dingemanse*,

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End Notes

[1] —An almost equivalent term is that of "Neo-Darwinism". In this book I use both terms as equivalent.

[2] —Dawkins (1976).

[3] —I shall always cite the scientific references I discuss in notes at the end of the book. Here, it is (Dias & Ressler, 2014).

[4] —Dias and Ressler (2014) shocked many evolutionists in showing that transmission of traits acquired during life is not limited to a few marginal cases of non-germline transmission of behaviour. I shall discuss in Chapter 8 how this study helped to make it impossible to keep on denying the existence of such processes.

[5] —Here the word is not taken in its religious meaning. It describes an idea that is strongly at odds with the most widely accepted view.

[6] —*Moshe* Szyf (2014) offers a clear diagram summing up the results of Dias and Ressler (2014).

[7] —Danchin (2021).

[8] —The website https://www.teteamodeler.com/ecologie/biologie/vivant/definition-vivant.asp defines life as "anything that can constitute itself by building its own living matter and **that is capable of reproduction** is alive; **life is transmitted**" (in bold what I underline, translated from French).

[9] —I have a good memory of this module because it was fascinating and because every time I arrived in class, I adopted a southern accent, so much so that my classmates thought I was from Marseilles.

[10] —We will see at the end of Chapter 11 that this largely accepted definition needs to be slightly improved.

[11] —The correct term here would be 'inclusive heritability' (see Glossary) because today the term heritability refers to the transmission **by genetic means only**. I therefore proposed the term inclusive heritability to depict parent-offspring resemblance, regardless of the mechanism (whether genetic or non-genetic) responsible for this resemblance (Danchin *et al.*, 2011; Danchin & Wagner, 2010).

[12] —This is the most widely accepted definition, although it is far too narrow (this issue is at the heart of this book).

[13] —Wang *et al.* (2017); Danchin *et al.* (2019b).

[14] —History is often quite unfair and does not necessarily remember the names of the real initiators of an idea. In this case, while *Charles Darwin* was writing his first book, he received a letter from *Alfred Russel Wallace* in 1858 in which the latter described the mechanism of natural selection that he himself intended to publish. Darwin then rushed to finish his 1859 book, "The Origin of Species", which became thee book that introduced the concept of natural selection.

-This change of term from 'transformism' to 'evolution' has a linguistic justification. It so happens that the French expression "*les espèces se transforment au cours du temps*", which gave the term transformism, translates into English as "species evolve in time", which therefore gave the term evolution. As the international language, which used to be French, was replaced in the 20th century by English, in the end it was the term evolution that prevailed. This is regrettable, however, because while the word transformism is neutral, the term evolution often implies an improvement, which would be incorrect in the context of biological evolution.

[16] —One usually cite Endler (1986) for having formulated this modern definition of natural selection, but in fact it was first formulated by Richard Lewontin (1970). This is known as the Lewontin's principle.

[17] —Ågren (2021) on page 86.

[18] —*Russell Bonduriansky*, for example, published an article in *Trends in Ecology and Evolution* with the explicit title "Rethinking heredity, again" (Bonduriansky, 2012).

[19] —A rough calculation leads to conclude that that very long span of time, saw between 400 and more than 1000 billion generations, a considerable transmission chain if ever there was one.

[20] —With 65 capital letters per Line and 35 lines/page or 2000 characters per page.

[21] —The 1962 Nobel Prize in Physiology or Medicine was awarded only to Watson, Crick and Wilkins for two joint papers (Watson & Crick, 1953; and Wilkins *et al.*, 1953). However, history has mainly retained Watson and Crick. At the very least, Maurice Wilkins, who was their co-recipient, should be added. Furthermore, two other important researchers made this discovery possible. First, this discovery is based on the two rules laid down by *Erwin Chargaff*. Secondly, it was *Rosalind Elsie Franklin* who first obtained X-ray crystallographic images of the DNA molecule and to deduce its structure. It was *Maurice Wilkins* who worked with her who then showed her images to Watson and Crick. However, as Franklin died of cancer in 1958, the Nobel Prize was only awarded to Watson, Crick and Wilkins, as the Nobel Prize is never awarded posthumously. *Rosalind Elsie Franklin* should have been associated with this Nobel Prize because of the leading role she played in this major discovery.

[22] —I introduced the term sequencic recently (Danchin *et al.*, 2019a), following its impromptu invention by *Hervé Philippe* during a heated discussion. I immediately adopted it because it perfectly describes the current use of the concept of genetics.

[23] —The second part of this sentence is not entirely true. For instance, alternative splicing between different messenger RNA sequences allows the construction of proteins whose complete sequence does not exist in one piece anywhere in the DNA. This phenomenon is particularly important in immunity for 'inventing *de novo*' a huge variety of antibodies, i.e. proteins each with a specific shape and therefore affinity to a given antigen (a molecule of infectious agents).

[24] —We propose this somewhat iconoclastic idea in the legend to Figure 7 of Danchin *et al.* (2019b).

[25] —This definition corresponds to what Richard Dawkins calls "*the genetic book of the dead*" in his book "Unweaving the rainbow" (Dawkins, 1998).

[26] —See Wagner and Danchin (2010) on biological information defining basic principles for studying biological information, and that proposes a classification of the various types of biological information.

[27] —I addressed the issue of the various levels of memory in Figure 7 of Danchin *et al.* (2019b). We will return to it in chapters 12 and 14.

[28] —See for instance Bonduriansky and Day (2018).

[29] —In Chapter 5 of his book *Unweaving the Rainbow* (1998) *Richard Dawkins* quotes a text by Charles Singer published in 1931 that leaves no doubt that even then genes were thought to be in the chromosomes.

[30] —Page 51 of Ågren (2021).

[31] —Page 12 of the original edition (Darwin, 1859).

[32] —Galton (1886). The origin of the term ‘regression’ comes from the observation that in this type of analysis, in the vast majority of cases, the slope of this relationship is significantly less than 1, which indicates that the offspring of tall people tend to be shorter than their parents, whereas the offspring of short people tend to be taller than their parents. Galton saw this phenomenon as a *regression* of the trait under study that naturally tend to regress towards the mean; hence the term regression. Furthermore, this phenomenon is at the origin of one of the most common errors in interpreting statistics, called 'regression to the mean', a subject that I have been examining in the context of behavioural heredity (see Danchin *et al.*, 2014; for more general considerations on the subject, see Stigler, 1999; Barnett *et al.*, 2005; Kelly & Price, 2005).

[33] —In the second half of the 20th century, statistical methods were developed to generalize the measurement of resemblance among all relatives. Parent-offspring resemblance should propagate along the family tree. Two cousins must therefore be more similar than two random individuals. Relatedness can then be incorporated into the same statistical model to measure resemblance. This has greatly improved the measurement of resemblance, i.e. heritability.

[34] —*Francis Crick* a formulé ce principe en deux fois, en 1958 lors du congrès de la Société de Biologie Expérimentale, puis dans un article remarquable de clairvoyance et de simplicité apparente (Crick, 1970; 1958). In the meantime, biologists had become accustomed to calling this major idea "the central dogma of molecular biology", so that this phrase naturally became the title of the 1970 paper.

[35] —The late *Richard Lewontin* provided an excellent critique of this attitude that leads to believe in one's dreams in a book full of infinite sarcastic wisdom (Lewontin, 2001). *Richard Lewontin* died in July 2021 after I finished the writing of this book.

[36] —For a reflection on causality, in particular the causality between DNA sequence and phenotypic traits (including so-called genetic diseases), see Lewontin (2001). One can also see https://aeon.co/essays/the-feedback-loop-is-a-better-symbol-of-life-than-the-helix.

[37] —Published in the journal Teaching Statistics (Matthews, 2000) to illustrate that a correlation cannot be interpreted as demonstrating causality. The best that can be said for such a correlation is that its existence would be expected if there were causality.

[38] —Modified from Matthews (2000).

[39] —Sentence from the abstract of Baron *et al.* (1987) who in March 1987 claimed in the journal *Nature* to have found thee gene for bipolar affective disorder on chromosome X, while in February of the same year Egeland *et al.* (1987) had claimed in the same journal to have found thee gene for this same disease "*at the end of the short arm of chromosome 11*". Yet both of these studies used very fine-grained data based on pedigrees. As *Richard* Lewontin (2001) asks on page 160, can such correlative data be trusted? This demonstrates how misleading purely correlative approaches in genetics can be and how they can lead to therapeutic dead ends.

[40] —Table 2 of Trerotola *et al.* (2015) lists cases where reputed ‘genetic’ diseases later proved to be at least partly inherited epigenetially. Their Table 3 lists example of cancer inheritance involving transgenerational epigenetic states.

[41] —Those who can read French can consult https://genetique-medicale.fr/la-genetique-l-essentiel/les-maladies-genetiques-les-plus-courantes-decryptees/article/six-exemples-de-maladies-genetiques-et-leurs-origines. After announcing the existence of 6,000 known genetic diseases, this site then develops only the case of 6 diseases for which the mutation-disease link is well established. It is likely that the vast majority of the 6,000 so-called genetic diseases identified worldwide are only qualified as genetic because the offspring inherit the disease from their parents (pre-DNA sense). There is no doubt, for example, that diabetes must be one of these 6,000 so-called genetic diseases. However, we will see that the high heritability of this disease is not based on sequencic variation. We will see throughout this book that the fact that a trait is transmitted is not sufficient to claim that the variation in this trait is of sequencic nature.

[42] —For more information https://aeon.co/essays/the-feedback-loop-is-a-better-symbol-of-life-than-the-helix or Wikipedia https://en.wikipedia.org/wiki/Wnt_signaling_pathway.

[43] —I am far from the first to argue that the ‘gene-for’-language is slippery. See for instance Bateson (1986); or Lewontin (2001).

[44] —Lewontin (2001).

[45] —I use here a metaphor that I published in 2013 in the journal *Trends in Ecology and Evolution* (Danchin, 2013).

[46] —In the Hindu religion the god Vishnu can take different material forms, or avatars. Similarly, in the Catholic religion Jesus Christ is an avatar (a material form) of god (an abstract entity). See Gilddon and Gouyon (1989).

[47] —Many authors have insisted that a gene must be defined in terms of information, for example Gilddon and Gouyon (1989) and Ågren (2021) for instance on pages 49, 63 and 77.

[48] —I proposed this as early as 2010 in an article published with my colleague Richard H. Wagner in the journal Oikos (Wagner & Danchin, 2010). Of course we were not the only ones to have this vision.

[49] —I made this proposal in Danchin and Wagner (2010), and then reiterated in Danchin *et al.* (2011).

[50] —As I like to give credit where credit is due, I would like to point out that this phrase 'inclusive heritability' was suggested to me by my Quebec colleague, *Luc-Alain Giraldeau*. Before that I was using the unsatisfactory term 'transmittability'. After listening to one of my lectures, Luc-Alain told me: "Why don't you call it 'inclusive heritability' as I suggested when reviewing your chapter 'Cultural Evolution' in the book we co-edited?" This was chapter 20 of the textbook *Behavioural Ecology* from Oxford University Press (Danchin & Wagner, 2008). When he told me this, I thought, "Eureka, that's the term I was looking for". Yet Luc-Alain had already suggested it to me a year before, and at that time I had not heard it.

[51] —Often also called "synthetic theory of evolution" or simply as I will often do "Modern Synthesis".

[52] —I will pass over acknowledged differences between these terms, because although these differences are real, they make sense in a historical context and are mainly a matter of nuance.

[53] —I discovered well after finishing the manuscript of this book that the phrase "Inclusive Evolutionary Synthesis" had been first used in 2004 by *Ralf J.* Sommer (2004) in the title of his review of Wallace Arthur's book entitled "Biased Embryos and Evolution" (Arthur, 2004).

[54] —This is exactly what Darwin's quote in Chapters 2 and 13 said.

[55] —Figure inspired from Danchin *et al.* (2019a).

[56] —For the equivalences between Weismann's 'germ plasm' and 'soma plasm', and the current terms genotype and phenotype, see Haig (2007).

[57] —Concerning the history of the genesis of this diagram see Griesemer and Wimsatt (1989).

[58] —Figures modified from Danchin *et al.* (2019b).

[59] —This well-known paper Tinbergen (1963) has been cited more than 4,300 times today, which is remarkable given the relatively small size of the scientific community directly concerned. In effect, while the logic of this paper applies to the evolution of any trait, Tinbergen focused his reasoning on the case of behavioural evolution. Despite this, the article applies far beyond the behaviourist community. *Nikolaas Tinbergen* was awarded the Nobel Prize for Physiology or Medicine together with *Karl von Frisch* and *Konrad Lorenz* in 1973. It should also be noted that Tinbergen's article had been preceded by an article by *Ernst Mayr* that clearly addressed the question of the different levels of explanation of biological phenomena (Mayr, 1961).

[60] —On this subject see the excellent book by *Nicolas Mathevon* (Mathevon, 2021).

[61] —Mayr (1961).

[62] —Mayr (1961).

[63] —These two fields of biology are so separate that they ignore each other and belong to two independent CNRS institutes. Mayr's Functional biology is the domain of the INSB (*INstitut des Sciences Biologiques*) while Mayr's Evolutionary biology is that of the INEE (*INstitut écologie et Environnement*).

[64] —More about TULIP that I co-directed from 2011 until the end of 2019 at: https://www.labex-tulip.fr/. I will return to TULIP in the final chapter of this book.

[65] —Chapters 2 and 3 of Dawkins (1976) define the concept of replicator (page 15 of the 2006 edition) and several definitions of the gene concept (Chapter 3).

[66] —On the question of whether genes are really replicators, see Chapter 5 of Lewontin (2001) starting on page 141. But this point is secondary to my purpose in this book.

[67] —*Dawkins* hesitated much about his title, as he recounts in the preface to the 30th anniversary edition of Dawkins (1976). Critics of the term saw the term selfishness (see Glossary) as a motivation, whereas genes have no motivation. However, the term is justified by the fact that it is genes that increase in frequency that persist, which by definition can only occur *at the expense of other genes*. As *Jarvid Ågren* (2021) says it (page 115): "*Selfish genes do not necessarily make selfish people*". More generally, a recurrent criticism of the evolutionary thinking is its use of concepts developed to describe all the subtleties of human behaviour, which is implicitly considered as fundamentally different from animal behaviour. However, it is disturbing that we are so reluctant to transpose to animals terms such as *altruism*, *selfishness*, or *interest*, or the notion of *cost and benefit*, or *cooperation*, *competition*, *bluff*, *cheating*, or *cuckoldry*... and the list could be much longer. It seems to me that these uses are fully justified because there is no factual argument that there was ever a discontinuity between our species and the rest of living beings. To think otherwise would deny the very existence of the continuity of life, and to replace it with a special phenomenon that occurred at the time of the emergence of our species and allowed the invention of a large number of phenomena as fundamental as those described by these very subtle words. This would imply leaving the realm of science, as there is not a single piece of evidence for such a discontinuity. Moreover, our everyday language has clearly incorporated the reality of the animal-human continuity as revealed by the phrase "it's human" that is commonly used to refer to our animality. You can check, but every time we use that phrase we can replace it without betraying the message by "it's animal". The phrase "it's human" is in fact used to acknowledge our animality and to claim that all these words that we invented to qualify our human behaviour in effect convey all the subtleties of our animality. They are designed to describe animal behaviour, human included. Finally, philosophically, since we cannot be judge and jury, we humans are the least well placed to judge any fundamental discontinuity between ourselves and the rest of the living world, and we should show a little more humility. There is therefore no reason not to transpose to animals the words so marvellous in their subtlety and precision invented to describe ourselves, unless we rebuff our animality and want to maintain a discontinuity between animals and humans, which would amount to denying the very existence of evolution.

[68] —I add the term 'so-called' because the content of this rule was never written by Weismann. What is called Weismann's rule is a later construction that was certainly inspired by the fact that he distinguished soma from germline, but that goes much farther than this single distinction.

[69] —This introduction is inspired from Danchin (2022a).

[70] —Exactly 12 236 967 SNP (Xia *et al.*, 2012).

[71] —The title of this chapter is taken from Maher (2008).

[72] —We saw in Chapter 2 how heritability can be estimated at the population level through statistical analyses of the trait value of parents and their offspring. We also saw how this statistical value obtained while ignoring all mechanisms of resemblance is nevertheless most often interpreted only in sequencic terms. There are heritability estimates for many, many traits such as human height, various human diseases (obesity, diabetes, heart disease etc.), behavioural disorders (autism, schizophrenia etc.), IQ and cognitive abilities, the tendency to leave the place of birth, the ability to resist parasites or other pathogens, various behavioural traits... The list could easily take up a full page of this book.

[73] —Since these initial results, further studies of the heritability of human height have led to heritability measurements by GWAS more similar to those obtained by conventional methods. However, the mismatch between these two methods is still much higher than expected and some studies strongly suggest that, at least for some traits, the mismatch will never be reduced to zero (e.g. López-Cortegano & Caballero, 2019).

[74] —Data from late September 2021. The big difference between these two estimates is that Web of Knowledge only counts scientific articles published in scientific journals indexed by the company, whereas Google Scholar counts all hits on the net, which is of course much larger. For example, it includes citations of books, which Web of Knowledge does not.

[75] —For example, the fact that SNPs usually have very few possible values (often 2, maximum 4) implies the use of statistical models with low statistical power and a very low capacity to test complex interactions at different scales in the genome. This can therefore lead to the failure to detect the participation of certain SNPs in the emergence of the studied trait, which would naturally lead to an underestimation of the genetic heritability.

[76] —This is one of the main points of Trerotola *et al.* (2015) who review the molecular mechansms involved in epigenetic inheritance. The abstract says "Epigenetic programs may account for a significant fraction of the 'missing heritability.' ".

[77] —Here I elaborate on ideas from Danchin (2013).

[78] —This attitude is rampant in the evolutionary literature. Ågren (2021), for instance alludes to it when he says on page 58 that "*In organic evolution, the role of replicator is usually played by genes*" or when, on page 62, he evacuates the question of non-genetic inheritance in a single sentence "*There is increasing evidence that parent faithfully pass on, more than genes*", or when on page 82 he says that critics of the gene's-eye view claim that "*genes play no special causal role and biology needs a more inclusive notion of inheritance*". In the same vein, Ågren (2021) only tackles the issue of the current calling for a new synthesis at the very end of his book on pages 188-190.

[79] —See in particular Nowak and Sigmund (1992) who shows that in a prisoner's dilemma game, the strategy called Generous tit-for-tat invades a population of individuals playing tit-for-tat. Generous tit-for-tat differs from tit-for-tat only in that in a few percent of the cases, these individuals cooperate following a defection of their partner. This few percent change the whole evolutionary destiny of the populations.

[80] —For the number of cell types among the 37 plus trillion cells in a human organism, see for example https://www.nature.com/scitable/blog/bio2.0/discovering_new_cell_types_one/. Estimates of the number of cells in a human body range from 100,000 to 30,000 billion cells.

[81] —Szyf (2014).

[82] —More in Danchin (2022b).

[83] —We will see later in this chapter that this definition is incomplete.

[84] —Some authors add a fourth component to epigenetics, that of the study of prions and chaperone molecules (Lindquist, 2011; Newby *et al.*, 2017; Halfmann *et al.*, 2012; Halfmann & Lindquist, 2010b; Shorter & Lindquist, 2005; Halfmann & Lindquist, 2010a; Saibil, 2013), which will be discussed in the third part of this book. This may make sense in particular in evolutionary approaches (Danchin, 2022b). However, such a broadening of the concept of epigenetics would be tricky as the memory and transmission properties of prions and chaperone molecules are probably too different from the three mechanisms classically included in epigenetics, so I would tend to treat them separately.

[85] —For a visualization of the different scales of cytosine methylation and histone modifications within chromatin one can for example see figure 1 of Rey *et al.* (2016).

[86] —For example Rebollo *et al.* (2012) page 22 that reports in a few words the history of this term.

[87] —Biémont and Vieira (2006) that reviews the evolutionary importance of non-coding sequences.

[88] —Note that these sncRNAs have all the characteristics of a hormone.

[89] —Pak and Fire (2007).

[90] —Bonduriansky and Day (2018) on page 45.

[91] —Ruby *et al.* (2006); Batista *et al.* (2008); and Wang and Reinke (2008).

[92] —Wang *et al.* (2017).

[93] —Wang *et al.* (2017).

[94] —Wang *et al.* (2017).

[95] —This is called "codon usage bias" (Frumkin *et al.*, 2018). See also Yang *et al.* (2019).

[96] —Yang *et al.* (2019).

[97] —Frumkin *et al.* (2018) was one of the first to document this important molecular process. See also Yang *et al.* (2019).

[98] —For the large number of tRNA documented modifications, see Figure 2 of Ranjan and Leidel (2019). See also Leppek *et al.* (2018) that focuses more on mRNA modifications.

[99] —Tanenbaum *et al.* (2015).

[100] —Gingold *et al.* (2014).

[101] —For cancer cell proliferation see Ranjan and Leidel (2019).

[102] —Ranjan and Leidel (2019); and Leppek *et al.* (2018) but there is a whole series of articles on the regulation of translation as a function of the environment e.g. following a thermal shock.

[103] —Project named "The 4D nucleosome project" (Dekker *et al.*, 2017).

[104] —I develop these ideas in Danchin (2022b).

[105] —It is because of this important characteristic that not all gene expression states fall within the scope of epigenetics. According to this widely accepted definition, changes in gene expression states of transient nature that occur at every moment of life do not fall within the domain of epigenetics. Here I could quote a very long list of articles. Here is a very small selection, preferably choosing review or opinion articles on the subject (Danchin, 2022b; Nicoglou & Merlin, 2017; Danchin *et al.*, 2019b; Ashe *et al.*, 2012; Rando & Verstrepen, 2007; Haig, 2007; Fablet & Vieira, 2011; Jablonka & Raz, 2009; Jablonka & Lamb, 2010; Akimoto *et al.*, 2007; Gupta, 2007; Morgan *et al.*, 1999; Henderson & Jacobsen, 2007; Richards, 2006; Bossdorf *et al.*, 2008; Jablonka & Lamb, 2005; Danchin *et al.*, 2011).

[106] —Cubas *et al.* (1999).

[107] —Cubas *et al.* (1999). These authors were interested in this particular case because it regularly showed spontaneous reversal to the snapdragon phenotype, suggesting that this non-Mendelian transmission must be based on something other than mutation in the classical sense. For ecological implications see Herman and Sultan (2011) and Richards *et al.* (2017).

[108] —Here the term heritability is used somewhat abusively when applied to multi-cellular organisms, as initially the term was coined to describe parent-offspring resemblance in general.

[109] —On this heated debate, see Grossniklaus *et al.* (2013). I myself have participated a lot in this debate, trying to always place them in the context of inheritance (Danchin *et al.*, 2004; Danchin & Wagner, 2008; Danchin & Wagner, 2010; Danchin *et al.*, 2011; Danchin, 2013; Danchin *et al.*, 2013; Danchin *et al.*, 2019b), as this is the central point for ensuring the transfer between the study of mechanisms taking place at the infra-individual scale [the functional biology of Mayr (1961)] towards the intergenerational or even evolutionary scales in order to unify these two major fields of biology. I also defended this last idea on several occasions (Pocheville & Danchin, 2015; Danchin & Pocheville, 2014).

[110] —However, we will see in Chapter 9 that perhaps these epigenetic marks do not in fact escape the waves of demethylation-remethylation occurring during meiosis but are in fact added to the gametes during their maturation. Such a mechanism would then have the advantage of targeting specific epigenetic mark.

[111] —On the question of the origin of epigenetic processes one can read Moore (2020) who suggest that epigenetics probably emerged at the same time as life, and may even have preceded the appearance of RNA and DNA. More generally the fact that epigenetic processes exist in all unicellular organisms strongly suggests that epigenetics existed well before the evolution of multicellularity about a billion years ago.

[112] —Here I am using the expression used by *Elizabeth Pennisi* (Pennisi, 2008). I belong to this scientific trend, and it is the ultimate goal of this book to help shape this new synthesis. This trend has more and more representatives all over the world. Among the pioneers I would mention *Eva Jablonka* of Tel Aviv University who has certainly been an inspiration for many of us. We can also mention *Massimo Pigliucci*, professor of philosophy at the City College of New York. I must also mention *Mary Jane West-Eberhard*, a member of the National Academy of Sciences of the USA, who undoubtedly played an important role as a precursor by pointing out the existence of various forms of non-genetic inheritance, even if at the time she did not use this expression (West-Eberhard, 2003). Finally, I must mention *Wallace Arthur* (2004)'s book. I will stop here as the list would be too long.

[113] —Champagne (2008).

[114] —E.g. Weaver *et al.* (2004). For a good review see Beery and Francis (2011).

[115] —Denenberg and Whimbey (1963).

[116] —E.g. Weaver *et al.* (2004). For a general view see Champagne (2008).

[117] —Francis *et al.* (1999).

[118] —Champagne (2008).

[119] —Modified from Champagne (2008).

[120] —For instance see the series of papers produced by Isabelle Mansuy's group in Switzerland (e.g. Franklin *et al.*, 2010; Bohacek & Mansuy, 2015; Franklin *et al.*, 2014; Franklin & Mansuy, 2010).

[121] —On the adaptive value of the transmission of maternal behaviour, see Weaver *et al.* (2004); and Beery and Francis (2011).

[122] —For the case of parental care transmission in humans, see Champagne (2008).

[123] —See McGowan *et al.* (2009).

[124] —Researchers have taken to referring to this model as the 'rat maternal care paradigm' (e.g. Beery & Francis, 2011), which is particularly useful for understanding the origin of these processes in humans.

[125] —See Champagne (2008) and the excellent article on the history of this approach (Beery & Francis, 2011).

[126] —Inspired from Danchin *et al.* (2019b).

[127] —Anway *et al.* (2005).

[128] —See for instance Kaiser (2014).

[129] —Kaiser (2014) reports on this controversy. However, there have since been so many publications detailing the molecular mechanisms involved in this type of process that this debate is no longer relevant.

[130] —See for example the documentaries by *Marie-Monique Robin* and her last book entitled "*La fabrique des pandémies*" Cahiers LIBRES La Découverte. This masterly work shows to what extent we are decoupled from the environment in which we live. A more inclusive vision of life in general seems to me particularly necessary to get out of this biased vision, which is worrying to say the least.

[131] —Guerrero-Bosagna *et al.* (2013).

[132] —Crews *et al.* (2007).

[133] —Skinner *et al.* (2011).

[134] —Kaiser (2014).

[135] —Santi *et al.* (2018) documents the links between outdoor temperature (this role has been known for a long time) as well as certain air pollutants, with a decrease in human male fertility. See two review articles, one on the effects of air pollution (Di Nisio & Foresta, 2019), and the other on the effects of water pollution (Jurewicz *et al.*, 2018). They conclude that while causality between pollutants like endocrine disruptors and male fertility is well demonstrated in animals, in humans the arguments remain only correlative for obvious reasons. It seems to me that the well-supported causality in animals can be extrapolated to humans, as this is done every day in medicine. One should not forget that the strategy of the chemical industry consists of producing articles claiming to be scientific and concluding that the concerned molecules are harmless. This dilutes the true scientific articles among numerous pseudo-scientific articles claiming the opposite, with the goal of maintaining as much doubt as possible. This strategy has been documented regularly since it was first implemented by the tobacco industry.

[136] —Dias and Ressler (2014). By the way, you will note that in Chapter 2 I argued that co-occurrence or correlation does not always mean causation. It is therefore amusing to see that animals use the co-occurrence between a thing and a danger to learn that the thing in question is dangerous. In nature this works very well. The wren parents I met on the moor were right to panic at my proximity to their chicks. In the lab, however, we can use this ability by artificially associating a benign thing or event with a real danger to study how this association is remembered and reused in the future.

[137] —See Chapter 3 for the difference between proximate and ultimate mechanisms.

[138] —Dias and Ressler (2014).

[139] —The genetics of rodent olfaction are well known [details in Szyf (2014)].

[140] —Note, however, that in this example transmission occurs through both the male and female routes (Dias & Ressler, 2014).

[141] —Dias and Ressler (2014); Szyf (2014).

[142] —In fact, we will see in Chapter 9 that this is not necessarily the case.

[143] —Aoued *et al.* (2019).

[144] —Although this question is still an enigma, as we shall see in the next chapter, knowledge on this subject has progressed a great deal (see for example Chen *et al.*, 2016b).

[145] —From Ost *et al.* (2014) which presents results in Drosophila (or fruit fly) very similar to those developed here. A more recent study reports that 39% of humans worldwide are overweight, with 13% classified as obese (Sun *et al.*, 2018).

[146] —See Chen *et al.* (2016a); and Sharma *et al.* (2016).

[147] —Chen *et al.* (2016a); and Sharma *et al.* (2016).

[148] —Chen *et al.* (2016a).

[149] —Reviews in Wang *et al.* (2017); and Zhang *et al.* (2018).

[150] —During gene expression, a messenger RNA carries a copy of the gene sequence to the cytoplasm where it is then translated into protein by the ribosomes. Transfer RNAs (tRNAs) have 76 to 90 nucleotides long. On one side they bind a specific amino acid, and on the other side they have a sequence called an anti-codon because it is complementary to the codon coding for this specific amino acid. This allows the concerned amino acid to be placed in the right place in the amino acid chain that makes up the protein being synthesised. Nice pictures of a tRNA can be found on Wikipedia (https://en.wikipedia.org/wiki/Transfer_RNA). These tRNAs can be transformed into a series of sub-sequences called tsRNAs, which play very important roles in particular in non-genetic inheritance.

[151] —As a reminder, histones are the proteins around which DNA is wrapped to form chromatin.

[152] —Grandjean *et al.* (2015).

[153] —Zhang *et al.* (2018), which has authors in common with the 2016 study in *Science* mentioned above.

[154] —Sharma *et al.* (2016).

[155] —Cited in Chen *et al.* (2016b).

[156] —Several studies have shown a similar role for small ribosomal or transfer RNA (rRNA and tRNA respectively) derivatives. Furthermore, human sperm cells are known to contain such rANR and tRNA derivatives (Zhang & Chen, 2020).

[157] —During mammalian pregnancy, embryos can be affected through simultaneous exposure, which we have seen, does not belong to heredity. Furthermore, pregnant females can and do shape the development of their embryos according to the environment they encounter during pregnancy. Such phenomena would therefore participate to the information transmitted by the ova, which would always make the results more delicate to interpret than in males who cannot influence their developing embryos beyond fertilisation. It could be argued, for example, that these are simple phenomena of simultaneous exposure occurring during gestation. It is to avoid this type of argument that most studies reported here tested the effect beyond F2.

[158] —An example where the environmental effects also go through the female pathway is given in the article with reference 59 in Zhang *et al.* (2019).

[159] —Sentence of the abstract of Zhang *et al.* (2018). Note here that the sequence of small RNAs leads to changes in the epigenetic state of specific portions of the DNA in the germline, which explains the transition from F0 to F1. These epigenetic states are then transmitted from F1 to Fn.

[160] —See Zhang *et al.* (2019); , and Zhang and Chen (2020) and the many references therein. Similarly, as early as 2006 Lopez-Rangel and Lewis (2006) talks of the “histone code”.

[161] —For example, Lopez-Rangel and Lewis (2006); Danchin *et al.* (2011); Chen *et al.* (2016b); Wang *et al.* (2017); and Danchin *et al.* (2019b).

[162] —Franklin *et al.* (2010) and Wang *et al.* (2017).

[163] —Tuesta and Zhang (2014).

[164] —Moore (2020).

[165] —As we have seen in this chapter (reviews in Zhang *et al.*, 2018; Zhang *et al.*, 2019; Zhang & Chen, 2020; Chen *et al.*, 2016a).

[166] —Ost *et al.* (2014).

[167] —Review in Wang *et al.* (2017)

[168] —For a review see Zhang *et al.* (2012).

[169] —Greer *et al.* (2011).

[170] —Klosin *et al.* (2017).

[171] —Ashe *et al.* (2012).

[172] —Devanapally *et al.* (2015).

[173] —Remy (2010).

[174] —Vastenhouw *et al.* (2006).

[175] —A review on the existence of multigenerational transmission of environmental effects in *C. elegans* is Minkina and Hunter (2018).

[176] —Sun *et al.* (2018).

[177] —In addition to all the examples developed in Chapters 6 to 9 and the many references to which I refer, there are many examples of acquired traits being passed on at least to F2 and Box 3 only alluded to a selection of them.

[178] —Chen *et al.* (2016b) Page 734.

[179] —First page of Zhang *et al.* (2019).

[180] —Inspired from Danchin *et al.* (2019b).

[181] —For a very profound and somewhat humorous view of the Weismann barrier concept, see Chapter 5 of Lewontin (2001).

[182] —As Chen *et al.* (2016b) underlines it.

[183] —Chen *et al.* (2016b) addresses this type of issue in his conclusion, page 741.

[184] —We shall see in Chapter 16 that this contradiction is only apparent, and that in fact one of the strengths of the Inclusive Synthesis of Evolution is that it generalises the Modern Synthesis of Evolution without contradicting it.

[185] —As a reminder, cytosine is one of the four nucleotides that make up the long double DNA nucleotidic chain. We saw in Chapter 5 that one of the main epigenetic modifications involves the addition of a methyl radical (formula CH_3) to certain cytosines. In mammals, the addition of methyl radicals to many cytosines of gene promoters decreases the expression of the corresponding gene(s). This can lead to the complete silencing of these genes.

[186] —This is the main thesis of Jones *et al.* (1992). We also develop this issue in Danchin *et al.* (2019b).

[187] —See for example again Jones *et al.* (1992).

[188] —Estimate made in Gorelick (2003). Trerotola *et al.* (2015) also provides a nice diagram of the metabolic pathways linking cytosine, methylation and thymine. It should be noted, however, that the entire literature on the link between epigenetic marks and mutations is based mainly on recurrent correlations obtained in the study of cancer, among other things, which on its own does not allow us to deduce that there is a direct causality between methylation and mutation. However, a number of arguments strongly suggest a causal relationship between the presence of epigenetic marks (and in particular methylations) and strong increases in mutation rates at different scales see for example Makova and Hardison (2015).

[189] —Danchin *et al.* (2019b).

[190] —Waddington's experiments have been often quoted and commented on but not often repeated (Waddington, 1959; 1942; 1953). They remain particularly relevant almost 70 years later and are in fact the focus of this chapter. For a review of this topic, see Crispo (2007).

[191] —A concept which at the time had not yet really emerged.

[192] —Danchin *et al.* (2019b). See note 212 later in this chapter that provides a rough calculation of the number of generations necessary to get real genetic assimilation.

[193] —*Mary Jane* West-Eberhard (2003) who undoubtedly was one of the main pioneers in the emergence of the new evolutionary synthesis. There is no doubt about it, and we should not forget that she did a lot of groundwork.

[194] —Here, I do not specify the word heritable because we always leave a doubt on its meaning, in the same way as we use the word genetic in one sense or the other without specifying it according to how it suits us.

[195] —The idea was to avoid at all costs that the environment could somehow participate in generating mutations, as this seemed to go against the principle that mutations are not directed by the environment in a way that allows adaptation.

[196] —However, we shall see in Chapter 16 that I have always had great difficulty with this notion of hidden *standing genetic variation*.

[197] —See Figure 2 of Danchin *et al.* (2019b).

[198] —For a review on transposable elements, see Rebollo *et al.* (2012) which also has the merit of presenting a chronology of the study of transposable elements.

[199] —This major discovery earned Barbara McClintock the Nobel Prize in Physiology or Medicine in 1983.

[200] —For more details see Rebollo *et al.* (2012); Tricker (2015); and the pedagogical Figure 2 of Marin *et al.* (2020).

[201] —Vieira *et al.* (1999)

[202] —From Vieira *et al.* (1999); Biémont and Vieira (2006). This last article proposes a table summarising what was known at the time about the place occupied by transposons in the genome of various species.

[203] —page 24 of Rebollo *et al.* (2012).

[204] —Biémont and Vieira (2006); Rebollo *et al.* (2012); Tricker (2015); Rey *et al.* (2016); and Marin *et al.* (2020).

[205] —See Rebollo *et al.* (2012) page 25.

[206] —For a more comprehensive review of this topic see Box 2 of Rey *et al.* (2016).

[207] —From Biémont and Vieira (2006) page 524. You will note that this factor is of the same order of magnitude as that given for the mutagenicity of epigenetic marks.

[208] —See for example Biémont and Vieira (2006). See also Tricker (2015) who provides a plant point of view.

[209] —See Rey *et al.* (2016), in particular its Key Figure.

[210] —See Rebollo *et al.* (2012); and Biémont and Vieira (2006), as well as Olivier Rey's paper, of which I am a collaborator (Rey *et al.*, 2020) and Marin *et al.* (2020), which involves several prominent members of the CNRS research group "Epigenetics, Ecology and Evolution" (for more information on this GDR see https://rtp-3e.wixsite.com/rt3e?lang=en). This is also the case for our paper (Danchin *et al.*, 2019b) on epigenetically-facilitated mutational assimilation.

[211] —We introduced the concept of epigenetically-facilitated mutational assimilation in Danchin *et al.* (2019b) where we discussed only the mutagenic role of epigenetic marks, the role of transposable elements in producing genetic variation being discussed in (Rey *et al.*, 2016). In this book (e.g. in Figure 22), I give this process a more integrative meaning involving both the intrinsically mutagenic role of epigenetic marks, and the role of transposable elements as a powerful generator of sequencic variation.

[212] —Some estimates of the mutation rate are of the order of one point mutation per DNA base every billion generations (i.e. 10^{-9}). Apart from the fact that there are several reasons to think that these mutation rate estimates are unrealistically low, such fidelity seems incompatible with the possibility of adaptation by the Darwinian mutation selection process. But suppose that in reality the point mutation rate is in the order of 10^{-6} to 10^{-7} (or once every million to ten million generations), an increase in the order of 10^4 would imply that it would take hundreds or even thousands of generations for the engraving in the DNA sequence to become effective.

[213] —Iltis (1983). That paper was one of the first to propose that epigenetics may play a major role in the domestication of organisms, in this case that of maize. Teosinte resembles maize but differs in several important respects in terms of yield, including the fact

that teosinte cobs have only one pair of small kernels that detach naturally, whereas maize has cobs with hundreds of large kernels that do not detach. This latter characteristic is very important for crop harvesting. This same characteristic played a role in the domestication of wheat and rice.

[214] —In this context, the term epigenetics meant an effect of the environment on the phenotype.

[215] —For instance Jablonka *et al.* (1995); Jablonka and Lamb (1995); Lachmann and Jablonka (1996). By speaking of "mutational assimilation" *Eva Jablonka* had introduced long before us the fundamental idea that environmental stress can generate new genetic variants, contrary to the taboo of Neo-Darwinism (Jablonka & Lamb, 1995).

[216] —In a paper entitled "Environmentally induced epigenetic transgenerational inheritance of sperm epimutations promote genetic mutations", *Michael Skinner* sets the stage by proposing that epigenetic marks in the germline may ultimately facilitate the appearance of mutations in the germline (Skinner *et al.*, 2015), which is the thrust of our proposal. I had not discovered thats publication at the time we published this mechanism, which is why we did not cite it.

[217] —Anastasiadi and Piferrer (2019) where the possibility that epimutations generated by domestication may induce mutations is just addressed as a mere speculation.

[218] —Darwin (1859).

[219] —This agency, commonly known as the ANR, which was set up under the presidency of *Nicolas Sarkozy*, is today the main potential source of funding from the French state for research in all scientific fields. There is a lot to be said about its functioning, but it at least has the merit of existing.

[220] —This expression is used many times by this author in two articles (Baldwin, 1896a; b).

[221] —In fact, as *Jarvid Ågren* (2021) clearly shows the reduction of inheritance to genetics had started decades before the discovery of the DNA, mainly under the influence of *Ronald A. Fisher* (1930).

[222] —Details of the localities involved and the invasion of much of the British Isles by this behaviour during the first half of the 20th century, see Fisher and Hinde (1949). This article can be downloaded from the British Birds website at the URL: https://britishbirds.co.uk/wp-content/uploads/article_files/V42/V42_N11/V42_N11_P347_357_A059.pdf. See also Hawkins (1950).

[223] —Sherry and Galef (1984); and Hawkins (1950).

[224] —At least 7 species including several chickadees seem to have developed this ability (Hawkins, 1950).

[225] —Hirata *et al.* (2008) provides a detailed account of this fascinating story. See also Avital and Jablonka (2000).

[226] —For recent studies see (Lynch & Baker, 1993; Lynch & Baker, 1994; MacDougall-Shackleton & MacDougall-Shackleton, 2001; Feher *et al.*, 2009; Fitch, 2009; Logue & Leca, 2020; Williams & Lachlan, 2022; Hyland Bruno *et al.*, 2021) among many others. For a review see Aplin (2019).

[227] —See a whole series of papers by *Andy Whiten*'s very productive group at the University of Saint Andrews in Scotland, for example (Whiten *et al.*, 1999; Whiten, 2017). Andy recently published a review on this subject (Whiten, 2021).

[228] —References are: chimpanzee (Whiten *et al.*, 1999; Whiten, 2017; 2011; Whiten *et al.*, 2005; Whiten, 2007), orangutan (van Schaik *et al.*, 2003), vervet monkey (van de Waal *et al.*, 2013), cetaceans (Whitehead, 1998; Allen *et al.*, 2013; Krutzen *et al.*, 2005; Kopps *et al.*, 2014), meerkats (Thornton *et al.*, 2010), and birds (Feher *et al.*, 2009; Aplin *et al.*, 2015). The two bird studies are notable for their experimental approach of the dynamics of transmission within a population, one on song (Feher *et al.*, 2009), the other on how to use an artificial food source (Aplin *et al.*, 2015). For insects, the most elaborate arguments to date concern the transmission within a hive of a new feeding behaviour in bumblebees (Alem *et al.*, 2016), or the social transmission of sexual preferences in the fruit fly (Danchin *et al.*, 2018). These two studies share the qualities of the two above bird studies. Two reviews that are already a bit old are also very useful (Avital & Jablonka, 2000; Danchin *et al.*, 2004). A recent one is Whiten (2021).

[229] —I use the word 'empirical' in its usual English meaning, which does not have the pejorative connotation that it has in many languages. Empirical evidence is evidence resulting from concrete and indisputable biological facts. In this sense, empirical arguments are complementary to theoretical arguments which are based solely on logical reasoning often involving a mathematical approach.

[230] —Feldman and Cavalli-Sforza (1989); Cavalli-Sforza and Feldman (1983); Cavalli-Sforza and Feldman (1981); Feldman and Cavalli-Sforza (1984); Boyd and Richerson (1983; 1985; 1988; 1995); Richerson and Boyd (2005); Henrich *et al.* (2008); and Boyd and Richerson (2009).

[231] —Laland (1994).

[232] —Boyd and Richerson (1985); Henrich and Boyd (1998).

[233] —This is the most commonly accepted definition that was proposed by Boyd and Richerson (1985).

[234] —Avital and Jablonka (2000) identified more than thirty definitions of social learning. Here, by social learning I mean any learning that is influenced by observation of, or interaction with, another animal (most often a conspecific) or with products of its activity (Heyes, 1994). In this definition the term 'learning' is taken in a very broad sense, which includes imitation, copying of another individual or any situation where the observation of another individual then modifies the behaviour of the observer.

[235] —*Audrey Dussutour* who is a CNRS researcher in Toulouse and a collaborator from Brussels working on the unicellular myxomycete *Physarum polycephalum* have shown that the temporary fusion between two individuals allows the transfer of a behavioural pattern learned by one of the two individuals to the other individual (Vogel & Dussutour, 2016). Some of their results even strongly suggest cultural transmission. For people who can read French I recommend her book "*Tout ce que vous avez toujours voulu savoir sur le blob sans jamais oser le demander* » published at *J'ai lu* in 2019. This short book says a lot both about Audrey's research and about the shortcomings of todays' research. Very informative.

[236] —It pioneered the study of animal culture in the field Andy Whiten *et al.* (1999).

[237] —Gibson and Höglund (1992); and Pruett-Jones (1992).

[238] —Or 'mate choice copying', but I think that the latter is not appropriate because, as we have made clear with my colleague *Richard H. Wagner*, we believe that what informs observer females is not so much the choice of demonstrator females as the performance of the male who has successfully attracted a female (Wagner & Danchin, 2010). In other words, females are selected to choose the males that are most successful with other females, with the choice of other females merely revealing male attractiveness (Wagner & Danchin, 2010; see also: Danchin *et al.*, 2020). This is why I prefer the phrase "mate copying" that focuses on the males.

[239] —We published several articles on this case study (Mery *et al.*, 2009; Monier *et al.*, 2019; Monier *et al.*, 2018; Nöbel *et al.*, 2018a; Nöbel *et al.*, 2018b; Dagaeff *et al.*, 2016; Germain *et al.*, 2016; Loyau *et al.*, 2012). See also the review (Varela *et al.*, 2018).

[240] —Danchin and Wagner (2010).

[241] —Danchin *et al.* (2011).

[242] —Danchin *et al.* (2018). See also the comment by *Andy Whiten* (Whiten, 2018).

[243] —The distinction between these two forms of mate copying was made by Bowers *et al.* (2012).

[244] —Between 1999 and 2018, I wrote, submitted and defended a good 50 projects to fund my research. Each one took me days or even weeks of work. Only two were successful. I was also involved in a good 30 other projects while not being the principal investigator. One was funded. When successful, I never got the full funding i needed, which meant that the objectives had to be scaled down. There is a lot to be said about the functioning of research institutions in France. I would summarise in saying that recruitment to get a position is extremely competitive, and that the strangest thing is that when you have succeeded in getting a position, you have a salary, which is far from negligible, but you have no funding to operate. Many, many excellent researchers around me spent decades without any funding, which meant that they couldn't do any research of their own. I even ended up writing a specific section on this subject in my annual reports to the CNRS, as this situation seemed to me to be a real waste, both financially and intellectually.

[245] —Good students are those who know how to contradict their supervisor and make their own decisions. This was the case of Susana.

[246] —Mery *et al.* (2009).

[247] —Dagaeff *et al.* (2016).

[248] —Modified from Danchin *et al.* (2018).

[249] —For more details on this series of experiments, see Danchin *et al.* (2018) that can be downloaded at (http://www.edanchin.fr/publications/).You will also note that this first criterion replicates that of Dagaeff *et al.* (2016), which replicated while greatly simplifying it that of Mery *et al.* (2009). Similarly, the tests of the following criteria included a control replicating this result. Thus, there is no longer any reason to doubt that *Drosophila* females can perform mate copying.

[250] —As well as in the articles cited in several of the previous notes.

[251] —Some of these experiments were published separately by *Sabine Nöbel et al.* (2018b).

[252] —Pat Bateson had a similar apparatus to study mate choice in quails that was dubbed the Amsterdam apparatus. Another term that was suggested to me was panopticon (https://en.wikipedia.org/wiki/Panopticon).

[253] —Modified from Danchin *et al.* (2018).

[254] —Fisher (1930).

[255] —For more details on this issue see Nöbel *et al.* (Submitted) that also presents a model to back up our argument.

[256] —There is a whole theoretical literature in human sciences on the importance of conformity for the emergence of traditions. However, these approaches adopted the same type of formalism inherited from Boyd and Richerson (1985), a formalism that only imperfectly describes the conformity we have described in *Drosophila*. As a result, these models could not be used to determine whether social learning in *Drosophila* could lead to the emergence of long-lasting traditions. Therefore, we had to incorporate a model of a type more representative of cognitive reality into our study to verify this central point.

[257] —For example Alem *et al.* (2016).

[258] —We started to address this issue in a recent article (Nöbel *et al.*, 2018b).

[259] —We started exploring these various issues in Nöbel *et al.* (Submitted), which is a review article on conformity in both humans and animals.

[260] —For example *Lucy Aplin's* paper in tits (Aplin *et al.*, 2015), *Sylvain Alem* in bumble bees (Alem *et al.*, 2016) or all the research in chimpanzee (Horner *et al.*, 2006; Whiten, 2017).

[261] —Nöbel *et al.* (Submitted).

[262] —Inspired from Danchin *et al.* (2019b).

[263] —Note that there are rare exceptions to the vertical transmission of genes, and examples are regularly published, in particular in plants where some gene transmission occurs horizontally across species. This is a rare phenomenon but sufficiently frequent to affect the evolutionary process over the long term.

[264] —Inspired from Danchin (2013).

[265] —Behaviour is usually defined as the set of decision processes by which an individual responds (or adapts) rapidly to its environment (a term that includes everything external to an individual). This definition implies processes taking place on a short timescale (from fractions of a second to minutes), which distinguishes behaviour from other forms of acclimatisation to environmental change that take place over longer timescales, such as phenotypic plasticity in general, of which behaviour is a very rapid form.

[266] —Theoretical considerations have led authors to note that the pace of cultural evolution may be often quite similar to that of genetic evolution (Franz & Nunn, 2009). We are therefore not the first to have suggested that cultural traditions could persist over very large timescales.

[267] —Haesler and Seehausen (2005).This study concluded, based on the fact that the estimated heritability of this trait was very high, that more than one gene was involved with no dominance effects.

[268] —I strongly recommend reading this second article (Verzijden & ten Cate, 2007) in comparison with the previous one, as it illustrates the extent to which we have boxed ourselves into a view of heredity that solely emphasizes sequencic information.

[269] —The reader interested by the emergence of this phrase can find information in note 53 above.

[270] —We shall see in Chapter 16 that this is an incorrect impression because far from challenging the Modern Synthesis of Evolution, the Inclusive Evolutionary Synthesis generalises and thus reinforces the Modern Synthesis of Evolution. We must therefore be wary of this kind of impression, which is unfortunately strongly maintained by some supporters of the modernization of the Modern Synthesis who often imply that the Modern Synthesis of Evolution is false. It is not false, it is simply incomplete and therefore lacks the explanatory power needed to, for example, support biomedical research.

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[271] —See Kaiser (2014) which explains how researchers who propose ideas that are considered heretical because they disagree with the majority view can then have their careers thwarted by conformist currents aimed at preventing them from getting funding and publishing their results. This can be very effective in delaying scientific advances.

[272] —Lamarck (1809). According to https://collections.nlm.nih.gov/catalog/nlm:nlmuid-101393446-img this image is free of rights today.

[273] —I have even had a reviewer of one of my articles comment: "Either the authors are idiots, or they play the idiots". I confess that it took me a good week to get over it, and to realise that the idiot was perhaps not the one we thought.

[274] —See for instance Salt (1979).

[275] —This question was discussed at least as early as 1972 in an article that dealt more with the relations between domains of science that concern different levels of organisation (Anderson, 1972).

[276] —Maynard Smith and Szathmáry (1995); and Szathmàry and Maynard Smith (1995).

[277] —We developed this argument in Danchin *et al.* (2011).

[278] —Dawkins (1976). In his chapter 11 Dawkins speculated on the potential existence of replicators other than the sequencic replicator, in particular what he called memes, which he argued would constitute the basic memory entities of culture. As such, that Chapter of The Selfish Gene can be seen as a precursor to the emergence of the new evolutionary synthesis.

[279] —Schmitz *et al.* (2011). See also Schmitz *et al.* (2013). These papers, alongside with many others, talk of single methylation polymorphisms (SMPs) to make a parallel with single nucleotide polymorphisms (SNPs). See also Klironomos *et al.* (2013) for a discussion on the consequences of this high fidelity of transmission.

[280] —See for example Franz and Nunn (2009) who suggest that the rates of evolution generated by cultural inheritance may be similar to those of genetic evolution, and Danchin *et al.* (2018) who suggest with a simple model reproducing a transmission chain that traditions could endure for tens of thousands of generations.

[281] —Gasparini *et al.* (2006); Gasparini *et al.* (2001).

[282] —This figure is partly inspired from Jablonka and Lamb (1995); Klironomos *et al.* (2013); Danchin *et al.* (2019a).

[283] —for instance see Danchin *et al.* (2018); as well as Franz and Nunn (2009).

[284] —On page 370 of Schmitz *et al.* (2011). See also Schmitz *et al.* (2013).

[285] —This is the main argument of Tricker (2015) in the case of the transgenerational epigenetic priming of plants, but his arguments hold for animals too.

[286] —Uller *et al.* (2015).

[287] —Spatial autocorrelation means that the state of the environment at point A predicts the state at point B according to its distance from point A. Temporal autocorrelation means that the state of the environment at a given point and time predicts the future state of the environment at that point. For example, there is a strong temporal autocorrelation of weather conditions over a one-year time step due to the fact that the earth is in exactly the same place in its rotation around the sun one year later. The climatic conditions are therefore strongly autocorrelated on a one-year time step.

[288] —Kuhn (2021). See also https://en.wikipedia.org/wiki/The_Structure_of_Scientific_Revolutions

[289] —It is this view of science that prevails today. I have referred to it many times in this book, for example by saying that the facts are stubborn.

[290] —For French reading people, an excellent account of this story can be found at https://www.pourlascience.fr/sr/histoire-sciencescommunication-des-neurones-la-volte-face-du-professeur-eccles-17764.php.

[291] —This is, for example, the thesis of Jablonka and Lamb in their book (Jablonka & Lamb, 2005) or in their book chapter (Jablonka & Lamb, 2010). This type of assertion is also found more or less clearly stated in numerous articles, such as Bossdorf *et al.* (2008).

[292] —For those interested in the issue of these controversies, see for example the virulent response (Dickins & Rahman, 2012) that was made to our article Danchin *et al.* (2011). Often, such controversies are based on a distorted reading of the other side's writings. We made a collective response to that article that, beyond being unnecessarily aggressive, we felt, did nothing to advance this debate (Mesoudi *et al.*, 2013). Another example of conversation between deaf people is the article published in *Nature* in 2014, that contains two columns, each ignoring the viewpoint presented in the other column (Laland *et al.*, 2014).

[293] —This is, for example, what each of the two columns in Laland *et al.* (2014) does.

[294] —I remind here that, as we saw at the end of Chapter 3, the Modern Synthesis of Evolution is the product of a collective effort. It is a set of broad principles on which most researchers agree. This book therefore aims to help in revising, extending and completing these major principles in order to bring out a new conceptual framework for studying life and its evolution.

[295] —It is not by chance, for example, that the definition of animal culture presented in the Chapter 11 starts with "*Animal culture is the part of phenotypic variation that is inherited by...*", which constitutes a quantitative genetics type of formulation.

[296] —In ecology, the movement of individuals between two breeding events is called dispersal (Clobert *et al.*, 2001). Geneticists use the term migration to describe this phenomenon, but ecologists use that latter term to describe seasonal movements between two living areas, the breeding grounds and the wintering grounds. I therefore use the term dispersal in the sense of ecologists.

[297] —It took me more than 8 years of submissions and rejections by journals to get this result published in 1998 (Danchin *et al.*, 1998). This article has now been cited more than 600 times.

[298] —For more details on phenotypic variance decomposition, see two papers in which we developed these ideas (Danchin *et al.*, 2011; Danchin & Wagner, 2010). Figure 16.B is modified from Figure 1 in Danchin and Wagner (2010), itself reproduced in Danchin *et al.* (2011).

[299] —For the various definitions of heritability see Danchin *et al.* (2011); and Danchin and Wagner (2010).

[300] —Shannon (1948).

[301] —For instance, the measurement of information by Kullback, or Shannon's quantum theory, which is a generalization of Shannon's theory. For those who can read French, on the issue of the different conceptions of information and their application to biology, I highly recommend reading the book Dessalles *et al.* (2016).

[302] —Danchin *et al.* (2004).

[303] —Wagner and Danchin (2010).

[304] —Here we were reasoning for all types of organisms, from unicellular organisms to organisms with a brain, all of which have sophisticated sensory systems that allow them to sense their environment in real time.

[305] —Crick (1970; 1958).

[306] —We proposed this formalism in Danchin *et al.* (2019b). The idea of using this formalism for information flows occurring at the level of the 3D structure was suggested by *Arnaud Pocheville*, a co-authors of that article. An excellent idea indeed.

[307] —Wang *et al.* (2017) develops this subject a little.

[308] —And whose arguments are developed in Danchin *et al.* (2019b).

[309] —For cellular memory, see Bonduriansky and Day (2018).

[310] —Modified from Danchin *et al.* (2019b).

[311] —I recommend the reading of Arthur (2004) as it provides a magisterial analysis of the importance of development as the main moment during which variation emerges, which is visualised here in arrow 5.

[312] —According to https://www.theguardian.com/news/2018/mar/26/the-human-microbiome-why-our-microbes-could-be-key-to-our-health.

[313] —For a review see Manjrekar (2017).

[314] —For more information see Lindquist (2011); Newby *et al.* (2017); Halfmann *et al.* (2012); Halfmann and Lindquist (2010b); Shorter and Lindquist (2005); Halfmann and Lindquist (2010a). This is an area entirely open to exploration in terms of the implications for inheritance.

[315] —Read Saibil (2013), a review on the state of knowledge on chaperone molecules. In fact, there seems to be little information on the role of chaperone molecules in inheritance, with research focusing on the role of chaperone molecules under environmental stress (e.g. heat shock). Same comment for Prions.

[316] —For an introduction to this subject, see Bonduriansky and Day (2018) which devotes a chapter to it.

[317] —One can read Brown and Bomberger Brown (2000) and our response to it almost 15 years later (Danchin *et al.*, 2014), as they illustrate important things. First, they illustrate the extent to which we systematically interpret parent-offspring resemblance in terms of sequencic transmission, when many other more straightforward mechanisms could be invoked to explain the resemblance. On the other hand, the article in the cliff swallows also illustrates the necessity to account a purely statistical phenomenon that was highlighted a very long time ago (Galton, 1886) and that can generate strong but spurious results especially in poorly designed experiments. In our response, we show that this statistical trap, classically called "regression to the mean", can explain the very high heritability obtained in experiments moving chicks between colonies of various sizes reported in the article in cliff swallows. Nevertheless, Brown and Bomberger Brown (2000) provides an excellent illustration of the phenomenon of habitat imprinting, with young birds tending to recruit preferentially to colonies of a similar size to their birth colony.

[318] —It is true that for several generations now the rural exodus has led many people born in the countryside to move to cities, but this type of movement results from strong social constraints, such as the greater job market in cities than in the countryside. Since this phenomenon is very recent, it is a kind of exception that proves the rule.

[319] —Concerning niche construction see Odling-Smee *et al.* (2003); Odling-Smee (2010); Odling-Smee and Laland (2011).

[320] —For a detailed description and presentation of intraspecific variation in bower structure, and the consequences for sexual selection, see Diamond (1986); Uy and Borgia (2000).

[321] —A picture I took in 1990 in the bush west of Cairns in Queensland.

[322] —See for instance Uller and Helanterä (2019).

[323] —The term "gene expression" here covers a much broader set of processes than the term epigenetics, which refers only to changes in gene expression that are at least transmitted across generations of cells, i.e. during mitosis. Epigenetics therefore covers only part of what is covered by the term "gene expression".

[324] —As a reminder, any entity able to make copies of itself is a "replicator". For a critical look at the idea that genes are replicators see Lewontin (2001).

[325] —It is this sentence that led to the concept of 'fitness', which we thus translated into French as 'aptitude' in the textbook we published in 2005 at Dunod (Danchin *et al.*, 2005) and that we then published in English at Oxford University Press (Danchin *et al.*, 2008b).

[326] —The word sequence appears 9 times between pages 22 and 29 of the 2006 edition which deal with the primary (sequencic) structure of DNA.

[327] —Definition attributed to Williams and Dawkins by *Jarvid Ågren* (2021) on page 48 when citing Dawkins (1976) on page 272-273 of the original edition. That definition is clearly of a sequencic, i.e. post-DNA nature.

[328] —This assertion is supported by reading Dawkins' other books. For example, chapter 5 of "Unweaving the Rainbow" (Dawkins, 1998) leaves no doubt that he has a purely sequencic view of the concept of a gene, as we all have and as I clearly adopt in this book.

[329] —For more details on these ideas see Danchin *et al.* (2019b).

[330] —However, I have to temper this statement somewhat because, as we have seen in Chapters 10 and 15 (particularly in relation to arrow 9), transposable elements can indeed produce both sequencic and epigenetic variation which, although usually hidden by epigenetic marks, is capable of being revealed by environmental stresses (see for example, Marin *et al.*, 2020; Vieira *et al.*, 1999; Rebollo *et al.*, 2012). However, even if such standing variation exists, the chances that it includes variants that are precisely adapted to the myriad of potential environmental changes seem very low to me.

[331] —Page 191 of the 2006 edition (Dawkins, 1976). In fact the actual citation started with “for an understanding of the evolution of modern man, we must begin by ….”. I purposely removed the words underlined here as, as I develop in note number 333 below, I am always extremely sceptical about arguments that claim that the case of the human species is original.

[332] —I have not developed the memetic approach at all in Chapter 11 because it does not seem to me to be appropriate for intergenerational studies, which is central to integrate cultural inheritance into the Inclusive Evolutionary Synthesis. The definition of culture presented in Chapter 11 seems to me to be a much more suitable alternative for the integration of cultural inheritance into

the new synthesis. However, for those interested I would strongly recommend reading Susanne Blackmore's book "The meme machine" which is absolutely outstanding (Blackmore, 1999).

[333] —As already said above, I am always extremely suspicious of any approach that assumes that humans are different, or apart, because in effect it amounts to asserting that humans are not governed by the same biological rules as the rest of the living world, which would mean leaving the realm of science. I intend to devote an entire book to this question. I will say no more about it here.

[334] —When I reread The Selfish Gene after I finished writing this book, I was struck by the fact that the only time I really disagreed with Dawkins (except for the two points made earlier in the text) was in his Chapter 11, which is also the most visionary. My reactions were to the fact that it assumed that humans are separate from the rest of the living world, and that the cultural phenomenon is only likely to affect the human evolution. These are both important points, but secondary to Dawkins' great insight in that chapter, which remains very impressive almost 50 years later.

[335] —In fact, if you were to ask a panel of evolutionary scientists you would find that the vast majority of them have forgotten the existence of the chapter on cultural replicators.

[336] —See for instance Section 2.4, or pages-188-189 in Ågren (2021). The risk of having gene-like prejudices when seeking other replicating entities is that it may make us oblivious of any other types of replicating entities.

[337] —This article shows that, in a number of situations, a cultural variant can be retained during evolution, even if this cultural variant significantly reduces the genetic fitness of the individuals carrying it (Laland, 1994).

[338] —See for example Marin *et al.* (2020); Vieira *et al.* (1999); Rebollo *et al.* (2012) for very informative reviews.

[339] —For this entire chapter I draw on Danchin *et al.* (2019a).

[340] —For a review see Zhang *et al.* (2019).

[341] —There are several reviews on this issue that is now well documented in a number of quite different organisms (e.g. Szyf, 2015; Sharma, 2015; Creemers *et al.*, 2012; Dorval *et al.*, 2013; Mitchell *et al.*, 2008; Wang *et al.*, 2017).

[342] —This corresponds to what Tricker (2015) mean when saying on page 2 that “epigenetic priming is targeted”. The underlying high specificity of the epigenetic priming is provided by the sequencic complementarity existing between the sncRNAs that transfer the environmental information and the target region (Tricker, 2015).

[343] —As we have seen this was demonstrated for example (Vastenhouw *et al.*, 2006) over 80 generations and (Devanapally *et al.*, 2015) over 25 generations in the worm *C. elegans*. A review article on this topic is (Remy, 2010).

[344] —The question of the existence of this third timescale is the topic of Danchin *et al.* (2019b).

[345] —Figure inspired from Figure 4 of Danchin *et al.* (2019a).

[346] —To my knowledge, the origin of the sncRNAs present in the microvesicles of the epididymis lumen involved in the inheritance of the effects of environmental stresses is not known (see Chapter 9). It could be either directly the brain-born sncRNAs, or secondary sncRNAs produced in the epididymis under the action of the brain-born sncRNAs.

[347] —In discussing the Weismann barrier concept, the late *Richard Lewontin* speaks of a "magic shield" against "hurricane forces" that "threaten precious DNA from the outside" (page 139, Lewontin, 2001). I usually talk about "a safe that even protects against nuclear explosions".

[348] —For an analysis of this phenomenon see Chen *et al.* (2016b), and in particular their box 3.

[349] —Fisher (1932).

[350] —Jablonka *et al.* (1995).

[351] —Danchin *et al.* (2019a).

[352] —Feng *et al.* (2010) page 627. See also Mirouze and Paszkowski (2011).

[353] —Jablonka and Raz (2009).

[354] —Methods have been proposed for making such comparisons, for example Danchin *et al.* (2013); Tal *et al.* (2010).

[355] —Inspired from Danchin *et al.* (2019a).

[356] —As illustrated for example in Rey *et al.* (2016).

[357] —As illustrated for example in Danchin *et al.* (2019b).

[358] —Figure adapted from Danchin *et al.* (2019a).

[359] —This idea is developed in Danchin *et al.* (2019a).

[360] —I take the idea of modernising the Modern Synthesis of Evolution from *Elizabeth Pennisi* (Pennisi, 2008).

[361] —See pp 8 of Ågren (2021).

[362] —This definition is modified from the first sentence of Alexander Bentley's paper Bentley *et al.* (2004). That paper tests one of the classic models of the Modern Synthesis called the Kimura neutral model explaining the distribution of genetic variants in a population on three types of human cultural data (pottery types, patent citations, and first names in the USA). The latter datasets had 6.3 million data points, a sample size that will probably never be reached in genetics. He finds that the distribution of these three datasets is exactly as predicted by the neutral model, showing that the logic developed to describe genetic variation in populations is perfectly suited to describe the distribution of other types of heritable variants such as cultural variants, a very interesting parallel.

[363] — As a reminder, the dichotomy I am making here between infra- and supra-individual biology corresponds exactly to the one Ernst Mayr made between functional and evolutionary biology (Mayr, 1961).

[364] —Ernst Mayr (1961) already commented on the consequences of that situation.

[365] —In France, this split is reflected, for example, within the CNRS (the continent's largest research institution) by the separation of biology into two institutes, one the Institute of Biological Sciences (INSB) and the other the Institute of Ecology and Environment (INEE). Far be it from me to regret the individualisation of the INEE within the CNRS, because it seems to me that historically this was an absolutely necessary step, but this separation should have be accompanied by a real incentive policy for collaborations between these two institutes, precisely in order to create the necessary context for the emergence of the Inclusive Evolutionary Synthesis. The least that I can say is that for the moment this is just wishful thinking on my part.

[366] —Heyer *et al.* (2005).

[367] —Laland *et al.* (2010) reviews the evidence that human evolution has been shaped by an interaction between genetic and cultural inheritance, the latter changing the selection pressures on genes. That paper includes a long list of genes whose pattern of variation in human populations has been shown to be influenced by cultural processes.

[368] —For example Rendell and Whitehead (2001); Whitehead (1998); Rosenbaum *et al.* (2002).

[369] —Rendell and Whitehead (2001); Krutzen *et al.* (2005); Kopps *et al.* (2014).

[370] —The late *Barry Sinervo*, for example, proposed this concept that brings together all the social dimensions of natural selection (Sinervo *et al.*, 2001).

[371] —This was published over 25 years ago (Laland, 1994). We have found a similar result in the context of mate choice in *Drosophila* (Danchin *et al.*, 2018), in particular through the integration of the Fisher runaway process discussed in Chapter 11. See also Nöbel *et al.* (Submitted).

[372] —This section is inspired by Bonduriansky and Day (2013).

[373] —The word lek means game in Swedish. A lek is a breeding system where males gather in a place (called a lek) and display to attract females who come to the lek to 'shop for genes'. Typically in a lek, one male gets almost all the matings, which should quickly reduce male genetic variation to zero, but this is not observed: this is the lek paradox.

[374] —Bonduriansky and Day (2013) provide a brief but comprehensive review of these mechanisms.

[375] —The idea that parasites are an essential source of phenotypic variation that play a major role in the evolution of their host constitutes one of the several major ideas that was developed by the late *William Donald Hamilton* (Hamilton & Zuk, 1982; 1989).

[376] —Bonduriansky *et al.* (2015).

[377] —Bonduriansky and Day (2018).

[378] —In Mayr's terminology (Mayr, 1961), the term functional biology corresponds in every respect to what I call infra-individual biology (molecular genetics, development, neurobiology, physiology…), and evolutionary biology to what I call supra-individual biology (ecology and evolution).

[379] —Zhang *et al.* (2019).

[380] —The sentence is on page 159 of Lewontin (2001), where he takes up a series of his texts older than 2001. The part concerned here was written in 1992. However, one could without hesitation replace '1992' in this quotation with '2022', as this statement is still fully valid today. In fact, since 2016 the last part of this statement is even truer than in 1992, because as we saw in Chapter 9 the heritability of this trait is probably not based on genetic variation at all.

[381] —Ost *et al.* (2014).

[382] —These diseases are called non-communicable because they do not result from the transmission of a pathogen (Gluckman *et al.*, 2009).

[383] —See for example de Rooij (2013); Franke *et al.* (2018).

[384] —See for example Painter *et al.* (2008); Veenendaal *et al.* (2013) who show that the grandchildren of women who experienced starvation during gestation showed greatly diminished health in adulthood, thereby showing that the deleterious effects of starvation lasted for at least two generations.

[385] —Bateson *et al.* (2004); O'Rourke (2014).

[386] —Review articles exist on this issue, for example Gonzalgo and Jones (1997); Plass and Soloway (2002); Sawan *et al.* (2008).

[387] —For example Beery and Francis (2011).

[388] —For example Ben-Ari and Spitzer (2010); and Ben-Ari (2008) which presents this view of the biology of the nervous system that extends and formalizes the ideas that led to the development of psychoanalysis.

[389] —In this section I use Nitschke *et al.* (2020) as well as Weiffenbach *et al.* (1998); Ritvo *et al.* (1985); Pellicano (2008); Kang *et al.* (2017); Bolte (1998); Ding *et al.* (2017); Sandler *et al.* (2000); Finegold *et al.* (2012).

[390] —Pellicano (2008).

[391] —The paper Ritvo *et al.* (1985) is a typical example of this kind of approach, which consists in interpreting transmission only in terms of DNA sequence. Moreover, in this data set, parent-offspring resemblance was not even significant, which did not prevent the authors from launching a sequencic interpretation by stating in the title that the gene(s) involved are probably autosomal.

[392] —This consortium, which involved no less than 138 authors, produced an article in the prestigious journal *Nature Genetics* (Szatmari *et al.*, 2007). The beginning of the abstract of this paper is typical of a purely sequencic view: "*Autism spectrum disorders (ASDs) are common, heritable neurodevelopmental conditions. The genetic architecture of ASDs is complex,…*" everything else is about genetic analysis and nothing else. No awareness that this heritability may have a genetic basis, *but also a non-genetic basis*.

[393] —Fraga *et al.* (2005) show in a large scale study of the DNA methylation and histone acetylation of monozygous twins that they are epigenetically indistinguishable during early years of life, but exhibit significant differences as they grow older.

[394] —The only form of non-genetic inheritance that largely escapes vertical transmission is cultural inheritance, which I therefore do not mention here, even though a substantial part of cultural transmission is vertical.

[395] —For instance Pellicano (2008).

[396] —Nitschke *et al.* (2020).

[397] —Bolte (1998). For this part I draw on (Finegold *et al.*, 2012) that recounts the beginning of this research approach.

[398] —Recent data show that up to 90% of autistic children have such severe and painful digestive disorders (Nitschke *et al.*, 2020).

[399] —Sandler *et al.* (2000). I saw some of the videos used to evaluate the effect of the vancomycin treatment and was struck by the incredible positive effect it had. During the treatment the offspring seemed to behave almost normally.

[400] —Some accused Ellen Bolte of being funded by the company that produces vancomycin, which is ridiculous when you consider what it means for parents to have an autistic offspring.

[401] —Kang *et al.* (2017); Ding *et al.* (2017).

[402] —Kang *et al.* (2017). That experimental study constitutes a remarkable advance in the possibility of 1- asserting a significant role of the intestinal microbiota in the development of at least some forms of autism, and 2- enabling the implementation of effective therapeutic protocols in the long term.

[403]—Review in Nitschke *et al.* (2020).

[404] —Antibiotics used during the first three years of life also appear to be involved in the development of autism by affecting the diversity of the microbiota (references in Nitschke *et al.*, 2020).

[405] —Pellicano (2008).

[406] —Riley (2016).

[407] —Blanchet *et al.* (2010).

[408] —See for instance Henley (2017); and Anonymous (2020). We did not manage to find scientific studies on that issue in spite of its importance for zoo conservation.

[409] —Nöbel *et al.* (2022).

[410] —Whitehead *et al.* (2004).

[411] —Laiolo and Tella (2005).

[412] —Laiolo and Tella (2007); Laiolo and Jovani (2007).

[413] —Jesmer *et al.* (2018).

[414] —Beautiful pictures of cranes in flight formation behind a microlight can be found on the internet.

[415] —For example the collective article Brakes *et al.* (2019).

[416] —The title of this last chapter was suggested to me by *Arnaud Pocheville*. It may seem pretentious, and I must therefore explain is logic. I do not intend to compare myself to Einstein, because contrary to what he alone brought to science, the setting up of the revision of the Modern Synthesis is the fruit of a collective input and not of a single person. Also, as the previous two chapters attempt to show, my hunch is that the changes brought about by the new synthesis will be as profound for the understanding of life as those brought about by the introduction of relativity in the understanding of the cosmos.

[417] —Many of the ideas developed in this last chapter are inspired from Danchin (2022a).

[418] —Adrian-Kalchhauser *et al.* (2020).

[419] —Edelaar *et al.* (2021).

[420] —It should be noted, however, that the Modern Synthesis did not really make such a mistake when it was established because in fact, at the time, sequencic was the only known pathway of inheritance. Contrastingly, today, to limit oneself to adding only epigenetics would indeed make this mistake because we know many other mechanisms of transmitted resemblance.

[421] —Adrian-Kalchhauser *et al.* (2021).

[422] —(Anderson, 1972; Salt, 1979).

[423] —This paragraph and the following are largely inspired from Danchin (2022a).

[424] —For more details about the links between the 3D or 4D structure of the DNA and epigenetics`, see Danchin (2022b).

[425] —Maynard Smith and Szathmáry (1995); and Szathmàry and Maynard Smith (1995).

[426] —This section is largely copied pasted from Danchin (2022a).

[427] —Danchin *et al.* (2005; 2008b).

[428] —about which I already had some knowledge Danchin *et al.* (2008a).

[429] —Danchin and Wagner (2008).

[430] —Danchin *et al.* (2004).

[431] —Mayr (1961).

[432] —see for instance: Danchin and Pocheville (2014).

[433] —The idea that we can black-box the details of the mechanisms of inheritance is rampant (although usually not spelt out) among tenants of the Modern Synthesis. For instance, in “The gene’s-eye view”, *Arvid J.* Ågren (2021) discusses that recurrent criticism made to the Modern Synthesis in his Chapter 2, and particularly in Section 2.2 (from page 46 to 53).

[434] —Mayr (1961).

[435] —For a more extensive comparison between these two rapid conceptual changes, see Danchin (2022a).

[436] —Which itself had given rise to the Modern Synthesis.

[437] —Boyd and Richerson (1985).

[438]—For an excellent discussion on this point, see Section 3.4 of Ågren (2021).

[439] —For more details on the different meanings of the concept of epigenetics see Danchin (2022b).

[440] —See Danchin *et al.* (2019a) that attempts to outline the basis of the Inclusive Synthesis of Evolution as described in this book.

[441] —According to Ågren (2021) on page 48.

[442] —Concept introduced by Conrad Waddington (Waddington, 1959; 1942; 1953).

[443] —Uller and Helanterä (2019), page 366. See also Arthur (2004).

[444] —Danchin and Wagner (2010)

[445] —Wagner and Danchin (2010).

[446] —The excellent work of Avital and Jablonka (2000) is on the ubiquity of animal traditions and the diversity of processes/conditions that can give rise to them.

[447] —This definition is by *Ernst* Mayr (1942).

[448] —Paley (1785). See also (Section 1.2 of Ågren, 2021).

[449] —Heyer *et al.* (2005).

[450] —More information on him at: https://www.persee.fr/doc/linly_0366-1326_1997_num_66_7_11182 or https://cths.fr/an/savant.php?id=106383.

[451] —More information on him: https://fr.wikipedia.org/wiki/Fran%C3%A7ois_Bourli%C3%A8re

[452] —More information on him at: https://fr.wikipedia.org/wiki/Robert_Barbault;