The role of non-genetic information in evolutionary frameworks

Katherine L. Moran, Yelyzaveta Shlyakhtina and Maximiliano M. Portal

Cell Plasticity & Epigenetics Lab, Cancer Research UK – Manchester Institute, The University of Manchester, Manchester, UK

ABSTRACT
The evolution of organisms has been a subject of paramount debate for hundreds of years and though major advances in the field have been made, the precise mechanisms underlying evolutionary processes remain fragmentary. Strikingly, the majority of the core principles accepted across the many fields of biology only consider genetic information as the major - if not exclusive - biological information carrier and thus consider it as the main evolutionary avatar. However, the real picture appears far more complex than originally anticipated, as compelling data suggest that non-genetic information steps up when highly dynamic evolutionary frameworks are explored. In light of recent evidence, we discuss herein the dynamic nature and complexity of non-genetic information carriers, and their emerging relevance in the evolutionary process. We argue that it is possible to overcome the historical arguments which dismissed these carriers, and instead consider that they are indeed core to life itself as they support a sustainable, continuous source of rapid adaptation in ever-changing environments. Ultimately, we will address the intricacies of genetic and non-genetic networks underlying evolutionary models to build a framework where both core biological information concepts are considered non-negligible and equally fundamental.

ARTICLE HISTORY
Received 30 November 2020
Revised 8 March 2021
Accepted 23 March 2021

KEYWORDS
Nongenetic; information; evolution; plasticity; heterogeneity

Introduction
The concept of evolution is central to the field of biology and yet it has been a subject of intense debate for centuries. With the discovery and increasing understanding of genetics, this theme became dominating in the evolutionary biology field and genetic information, encoded by the DNA sequence, became the key player in evolutionary theories. However, it is now coming to light that other factors also show the potential to contribute to evolutionary processes. Importantly, recent research into nongenetic information and its carriers has led to the suggestion that this plays a significant role in evolution.

This review will focus on the potential role of nongenetic information in evolution, whilst also expanding current frameworks and concepts surrounding this form of information and its transfer.

Historical concepts
Evolution through the years
The concept of evolution can be traced back thousands of years and since then has been a subject of intense debate, not just in the scientific community. This has produced a plethora of ideas, theories and inevitably, a significant amount of confusion and misconception surrounding this broad field. For the purposes of this review we aim to discuss evolution mainly with its core concept in mind, unaffected by the debates which remain ongoing to this day. Nevertheless, to provide context to some of our ideas we will briefly discuss how the concept of evolution has changed throughout history and how this has led to the understanding we have today.

The current definition of evolution is a change in the characteristics of a species over time. Interestingly, this core concept can be identified in ideas dating back to the first evidence of evolutionary theories found in ancient Greece (Figure 1). The Greek philosopher Anaximander (c. 610 – 546 BC), when speculating on the origins of man, theorized that life originated from other lifeforms and in doing so inferred that organisms change to become different types of organisms over time (discussed in (Trevisanato 2016)). A key observation leading him to this conclusion was that human children require parental care. He reasoned that this provided evidence that humans could not have
appeared on Earth in their current form, they simply would not have been able to survive the early stages of life. Therefore, humans must have come into being via a series of changes from a different creature, once this creature had reached an age at which humans no longer required care. Anaximander’s thought process is evidenced in writings of Plutarch: “… He [Anaximander] says that in the beginning man was born from creatures of a different kind because other creatures are soon self-supporting, but man alone needs prolonged nursing. For this reason, he would not have survived if this had been his original form…” (Kirk and Schofield 1983). Strikingly, these ancient ideas have remained at the core of evolutionary theory until the modern day.

The origin and nature of these changes was never found to be addressed by Anaximander himself, however numerous explanations were put forward in subsequent years. A notable example of this is found in the work of Pierre-Louis Maupertuis in the 1700s, who suggested that the appearance of a new species could be attributed to “mutant particles” or errors, implying that the changes observed over generations were driven by random events within an organism (Maupertuis 1751b). These ideas were derived primarily from his interests in dog breeding, in addition to observations of human defects displaying intriguing patterns of heredity such as a family containing a number of members with six digits (Glass 1955). From this, he extended his theory to describe how heredity is reliant on parentally derived “particles” and if by chance there is a deficiency or excess of certain “particles” then the offspring will present with a lack or excess of certain features. Importantly, his theory also predicted that errors or alterations of these “particles” could result in the production of a new species. In the words of Maupertuis himself, “each degree of error would have produced a new species; and by reason of repeated deviations would have arrived at the infinite diversity of animals that we see today” (Maupertuis 1751b; Focher 2014).

In contrast to this, a number of theories emphasized an important role for the environment in the evolutionary process, albeit in different ways. In that regard, the inheritance of acquired characteristics was a theory put forward by a number of people and was utilized in many evolutionary frameworks. This included the case of Jean-Baptiste Lamarck, to whom the idea is often unrightfully credited to, despite its appearance in theories many years prior to his. Importantly, it is not disputed that he did indeed use and accept this concept in his beliefs and teachings; however, it is thought he viewed this as common knowledge and never claimed the theory as his own. Nevertheless, the key premise of the inheritance of acquired characteristics describes how the environment can induce change and variation in organisms through use and disuse of physical characteristics, which is then inherited by the offspring. Interestingly, the environment is therefore identified as the driver of initial variation in a population which subsequently leads to long term change. This idea was based on numerous observations in nature, most notably examples in which the offspring presented enhanced features corresponding to those which were used favorably by the parents. Since this implies that parental experience can affect the offspring it was met with considerable controversy and was seen to directly oppose other popular theories, such as that of Charles Darwin, which identifies the environment-phenotype interaction to instead act on the variation already present in a population to drive evolutionary change (Darwin and Kebler 1859). Darwin’s central idea followed this logic, outlining the process of “descent with modification” (evolution) through natural selection (Darwin and Kebler 1859). This was based primarily on observations made during his voyage on HMS Beagle, most notably his experiences whilst visiting the Galapagos Islands involving the diverse species of finch and tortoise present there. Importantly, he identified different species of finch uniquely adapted for each individual island, and began to wonder if this was a result of changes in the populations over time driven by how well they fitted the specific environments they inhabited. From this, he developed his theory of evolution by the mechanism of natural selection, which he proposed could explain the adaptations he saw on these islands. Specifically, he theorized that phenotypic variation within a population would give rise to differential survival in a particular environment, with only the fittest able to survive and reproduce, leading to a change in the prevalence of characteristics in the population and, ultimately, evolution. Although Darwin’s early work gave the most complete evolutionary theory of that time, it was lacking in certain areas. He was unable to provide an explanation of how variations between organisms initially appeared or propose a mechanism of inheritance. To address this, he later published his theory of Pangenesis (Darwin 1868), involving the inheritance of gemmules (small particles capable of being modified following environmental change) through the germline. This will be discussed further in a later section alongside the inheritance of acquired characteristics.

Upholding Darwin’s evolutionary theory in combination with an understanding of Mendelian genetics,
Figure 1. The concept of evolution over time. From ancient Greece to the modern day, the core concept of evolution – change over time – has remained constant. Key historical ideas regarding evolution are shown in the lower panels accompanied by significant scientific developments in the upper panels for context.
which were the prevailing and widely accepted theories at the time, the modern synthesis was formed in the mid 1900s. This provided a number of postulates giving a complete theoretical framework surrounding the concept of evolution; mutation leads to phenotypic variation upon which selection can act and this in turn results in population level changes over time. It maintains the view that the environment-phenotype interaction largely acts at the point of selection rather than inducing the initial variation, as was proposed in earlier theories. Although widely accepted in the field, there have been recent calls for an update to the modern synthesis, including the formulation of an extended evolutionary synthesis (see Laland et al. 2014) for a full debate). This places increased emphasis on a number of additional concepts stemming from a broad range of scientific fields, notably the role of developmental processes and nongenetic information in evolution. Interestingly, a number of these concepts argue for an updated view on the function of the environment-phenotype interaction, which will be discussed in detail below.

Overall, it is clear that the core concept underlying evolutionary theory - change over time - has persisted from the time of its initial conception (Figure 1). The specific details of theories put forward since then have often reflected the research and knowledge present at that time, nevertheless, remarkable parallels with modern theories can be drawn from ideas presented centuries ago.

Despite the considerable leaps in the understanding of evolutionary theory in recent times, there remain a number of common misconceptions and inaccurate assumptions which interfere with the ability to fully appreciate the ideas surrounding these concepts. A key example of this is the misconception that the evolution of a species equates to an increase in complexity, or that evolutionary processes have directionality toward an ultimate goal. These ideas have been prevalent throughout history; however, they bring us to an important point. Evolution is simply understood as the change in a species over time, and does not necessarily mean that this change will result in increased complexity or proceed in any predetermined direction. This is not to say that evolution does not act in a certain direction, as directional selection can certainly occur resulting from a particular selective pressure. However, evolution can often be seen to "fluctuate in direction", demonstrating that the apparent direction of an evolutionary process may be subject to change rather than remaining fixed, particularly in response to fluctuating environmental conditions which results in variable selective pressures (Gibbs and Grant 1987; Bell et al. 2006). In view of this, it is therefore misleading to assume a fixed directionality when considering evolutionary processes.

Misconceptions regarding complexity and directionality, which have occurred throughout history, were likely influenced by other theories and ideas at the time, including religious beliefs. When the concept of evolution was relatively young there was little understanding of the life history of the species observable at that time. Furthermore, hierarchical views were widespread in many cultures leaving many people to place man above other lifeforms, judging by its apparent complexity and intelligence. Often, those proposing evolutionary theories were also respected in the field of species classification, making it plausible that elements of evolutionary theories were influenced by views on classification systems. Evidence of this can be found in texts from ancient Greece shortly after the concept of evolution began to be discussed. Aristotle (384 – 322 BC), a prominent Greek philosopher, formulated a Scala Naturae, or 'ladder of nature', classifying lifeforms based on increasing complexity, culminating in man’s superior position at the top (Aristotle 1910). Reflecting the limited knowledge and methodologies employed at the time, the grades were based on direct observations of organisms, principally the form of the young when birthed. His scala naturae even included minerals, albeit in the lowest grade beneath plants. Interestingly, Aristotle himself rejected the idea of evolutionary change in species, maintaining the view that his Scala Naturae was static and that there existed a final cause for all organisms based on his observation that species possessed specific features which fitted their function. Nevertheless, it is evident that his views, along with similar ideas from others, influenced theories of evolution for thousands of years. A key example of this comes from Lamarck, whose theory shows strong links to the classification system postulated by Aristotle despite there being a remarkable 2000-year gap. Lamarck's ladder of progress shows structural parallels with the Scala Naturae, yet he also introduces a directional adaptive force moving species upwards whilst concurrently generating an increase in complexity (Lamarck 1815). Mechanistically, this adaptive force is driven by his theories involving use and disuse and the inheritance of acquired characteristics. Importantly, this linear progression of species toward higher complexity was rejected by Darwin in his 'i think' sketch, illustrating his support for a branching model of evolutionary history which has prevailed in the field ever since.
Moving back to ancient Greece once again, it is clear that religious and philosophical beliefs also influenced the theories of Anaximander. At that time a number of philosophers spoke about ideas which held water in high regard and Anaximander was no different, theorizing that water was the origin of life. In addition to this, he lived surrounded by animals farmed for food, and made observations about fetuses from a variety of different species. From this, he reasoned that all animals resembled fish at early developmental stages, leading him to hypothesize that all species originated in water and some simply developed for longer to reach a more complex stage which enabled the move into drier environments. This example illustrates a further point, that often evolutionary theories were reflective of the extent of experimentation and research available at that moment in history. Simple observations of the lifeforms available to the individual at the time were commonly relied on in early evolutionary theories, whereas progression in understanding and experimental potential contributed hugely to the insight that was realized in later years. Regardless, it still remains remarkable, when comparing evolutionary theories spanning thousands of years, that a great number of parallels can be drawn despite the vast differences in technology and knowledge available to theorists. A similar misconception is that evolutionary processes will always result in beneficial changes for the organism, again inferring some sort of directionality over time. Evolutionary changes can, and often do, improve an organism’s survival within its niche; however, in this case the evolutionary process would be referred to as adaptation. Adaptation is an evolutionary process leading to an organism becoming better suited to its environment, and yet it is often used interchangeably with evolution. It could be argued that evolution is a process of changes in a species over time, whereas adaptation is a process of changes which make a species better suited to its environment. Thus, adaptation has increased directionality as opposed to evolution in general which can describe changes which have a neutral or even negative effect on an organism’s survival. Importantly, neither of these processes necessarily lead to an increase in complexity of a given species. There is a wealth of literature discussing the theories and details of these concepts; however, for the purpose of this review we will use these concepts as described here.

As explained above, the modern synthesis emphasizes Darwin’s theory of natural selection as the predominant mechanism driving evolutionary change. Darwin himself was convinced “that natural selection has been the main but not exclusive means of modification” (Darwin and Kebler 1859). One of the most heavily debated areas of evolutionary theory has been the identity of the underlying mechanism, with countless theories proposed throughout history surrounding selection and many containing elements of what we now know as natural selection. The Greek philosopher Empedocles (c. 494 – 343 BC) postulated that the Earth was once inhabited by many unusual creatures, such as cattle with human heads, but over time these creatures suffered extinction leaving the species he could observe in his day. This is thought to be the earliest indication of an idea similar to natural selection, as it suggests that certain organisms are subject to an increased probability of death due to particular suboptimal features. However, unlike natural selection, Empedocles’ theory does not depict this process of selection as the driving force behind evolutionary change in the species which do survive, instead he explains evolutionary change simply as the disappearance of certain maladapted species. Interestingly, there are numerous other examples of theories indicating that the concept of natural selection was being considered as a driver of evolution. In particular, one of Maupertuis’ key theories is often regarded as a precursor of Darwin’s natural selection. Specifically, he suggested that only adapted species have survived to the present day, inferring that evolutionary changes have resulted from some species having an adaptive advantage over others leading to differential survival and reproductive success (Maupertuis 1751a).

Following the publication of Darwin’s theory, natural selection has become widely accepted as a mechanism driving evolution. However, a widespread misconception is the idea that natural selection is the only mechanism able to produce evolutionary change, an idea which has been increasingly disputed in recent years (Huxley 1942; Kimura 1979). A number of additional mechanisms have been put forward including genetic drift which can, like natural selection, result in changes in characteristics over time at the population level. Genetic drift describes a change in frequency of a gene variant in a population which is independent of the associated phenotype and fitness. Importantly, it is based purely on stochasticity and chance. It is therefore equivalent to random sampling, and it could be said that natural selection is simply a bias in this sampling process. It follows that genetic drift and natural selection act concurrently, and genetic drift always acts. The effects of genetic drift are strongest in small populations, and consequently many observations of this process have occurred after population bottlenecks or the colonization of new environments by a subgroup of a
population (the Founder effect) (Goldblatt et al. 1992; Arcos-Burgos and Muenke 2002). This exemplifies the importance of considering multiple explanatory mechanisms when thinking about evolution, rather than remaining limited to natural selection only.

**Concept of nongenetic information**

As discussed above, there is increasing pressure on the field of evolutionary biology to reassess the postulates held within the modern synthesis framework in order to acknowledge a number of additional contributing factors to the process of evolution. Notably, one of the proposed changes argues that an increased emphasis should be placed on the contribution of epigenetic/nongenetic mechanisms. The aim of this review is to assess the role of these in evolution, hence we will begin with a brief discussion surrounding the concept of nongenetic information.

The term epigenetics was first coined by Conrad Waddington in 1942 as “the branch of biology that studies the causal interactions between genes and their products which bring the phenotype into being” (Waddington 1942b). Waddington predominantly worked in developmental biology, yet contributed significantly to a number of scientific fields during his lifetime, and was particularly fascinated by the relatively new field of genetics. At the time, two predominant theories of development prevailed; preformation, which claimed that organisms exist in their full form prior to development and require only time to grow, and epigenesis, the theory that organisms develop over time from an undifferentiated mass involving interactions between developing tissues. Importantly, Waddington rejected the notion that these theories are mutually exclusive, and from here derives his definition of epigenetics. Combining the two ideas, he postulated that organisms begin development with certain preformed characters (genetics) which, during development, interact to produce the final form in processes of epigenesis (the study of which he termed epigenetics). Essentially, he infers that epigenetic mechanisms enable cells equivalent to each other to diverge, producing variation between cell phenotypes which contributes to the development of a multicellular organism. However, it is important to note that the research atmosphere at the time was considerably gene-centric, owing to the growing popularity of the relatively new field of genetics. It is thought that this contributed to the way in which epigenetics was defined, despite very little being understood about gene function and regulation.

Since the original definition in 1942 the field of epigenetics has grown enormously, and with that came the concurrent growth and expansion of the usage and application of this concept. Interpretation of the term epigenetics has varied hugely, especially across different fields, resulting in confusion and discrepancy when epigenetic processes are reported in the literature (Deans and Maggert 2015). These inconsistencies are likely a reflection of the speed at which this term spread into a wide variety of scientific fields without a solid foundation of knowledge in this area to begin with. More recently, the term epigenetics has become heavily associated with chromatin modifications, particularly DNA methylation and histone marks. Consequently, it seems that upon mention of the term epigenetics, the immediate association is to a narrow field comprising only these modifications and its broader meaning has become limited in people's minds. Nevertheless, we would argue that the broad definition originally proposed by Waddington still stands and that, as discussed for the concept of evolution, the core concept has not changed over time despite the shift in people's perception. In an effort to avoid these common associations, which could prove limiting when conveying our ideas, we will use the term nongenetic in place of epigenetic. This will hopefully reduce the risk of presumption that our ideas are only referring to the limited repertoire of what has become known to be an epigenetic mechanism. We maintain that the original ideas surrounding the term epigenetic encompass the whole breadth of what we define to be nongenetic. Specifically, we will explore nongenetic information carriers in a broad sense, including any cellular information carriers distinct from the DNA sequence itself. For instance, non-coding RNA, protein folding, protein modifications, in addition to DNA methylation and histone modifications (Figure 2(A)). Importantly, these all have the potential to encode information which can influence gene expression downstream and therefore encode information about cellular phenotype. This satisfies Waddington's original concept since this information, which is independent of DNA sequence, could produce alternative phenotypes in isogenic cell populations (Waddington 1942b). Notably, the nature of these information carriers and their positions in signaling pathways often enables them to be modified by external stimuli, providing them with the potential to encode information about the environment.

In summary, we consider nongenetic information carriers to encode information, potentially related to environment stimuli, whilst remaining independent of
**A Carriers of nongenetic information**

- Histone modifications
- DNA methylation
- Protein modifications
- RNA
- Metabolism
- Cytoskeleton
- Prions

**B Properties of information carriers**

**Genetic**
- Changes are slow
- Changes are not easily reversed
- DNA is replicated prior to mitotic division and inherited symmetrically
- Information is only encoded in the DNA sequence
- Changes are usually not downstream of an environmental stimulus

**Nongenetic**
- Time scale
- Reversibility
- Asymmetric segregation
- Range of carriers
- Environmental influence

**C Contributions of nongenetic information to phenotype**

1. **DNA methylation in diatoms**
   - Maumus et al. 2009
   - DNA methylation levels:
     - Normal conditions: HIGH
     - Nitrate limited: LOW
     - Transposon expression:
     - Normal conditions: LOW
     - Nitrate limited: HIGH
   - Potential evolutionary implications:
     - Increased genetic and phenotypic diversity
     - Increased ability to respond to changing environmental conditions

2. **[PSI+] prion in yeast**
   - True et al. 2004
   - True & Lindquist 2000
   - Environmental change
   - [psi-] to [PSI+]
   - Genetic variation generated without selective pressure is expressed
   - Novel variation could provide an adaptive advantage in novel environments

3. **tsRNA in sperm**
   - Chen et al. 2016
   - Normal diet
   - Normal zygote
   - HFD
   - Normal zygote
   - Genetic variation
   - F1
   - Metabolic disorders
DNA sequence. This concept bears intriguing parallels with ideas discussed above, namely Lamarck's theory of evolution involving the inheritance of acquired characteristics and Darwin's theory of pangenesis. Lamarck's theory of evolution, when taken at face value, would be ridiculed and rejected in modern times just as swiftly as when it was first published. The often-quoted example describing the lengthening of a giraffe's neck, which was used to illustrate his idea of use and disuse, seems nonsensical in light of modern understanding and knowledge. Not only would this require transfer of information from the soma to gametes, it suggests the existence of a mechanism which can interpret this information to target specific genes in order to alter the offspring's phenotype in a defined way. It has been argued that this is a fundamental problem with Lamarck's theory, one for which a solution is inconceivable even in the modern day. However, as will be stated throughout this review, we maintain the view that absence of evidence cannot always be taken as evidence of absence.

Nevertheless, the general principle underlying Lamarck's theory, that the environment can influence an organism in a heritable manner which provides an adaptive advantage, could almost be considered a foreshadowing of nongenetic information since it predicts the existence of heritable factors which are modified by environmental stimuli and manifested in the offspring's phenotype.

All too often, teaching of evolutionary theory takes the standpoint that the inheritance of acquired characteristics, including Lamarck's theory of evolution in which it is used, directly opposes the theories and ideas of Darwin. What is frequently unappreciated is the fact that Darwin was in fact a supporter of Lamarck and the inheritance of acquired characteristics. In his book On the Origin of Species he specifically pinpoints the inheritance of acquired characteristics as a source of variation upon which natural selection can act (Darwin and Kebler 1859). Darwin even proposed his own theory of pangenesis to provide a mechanism for this process (Darwin 1868). Pangenesis posits the existence of gemmules that transfer information from the soma to the germline and therefore provide an explanation for the inheritance in the aforementioned theories. Importantly, this theory argues for the existence of information carriers which can be modulated by environmental stimuli and also influence phenotype. Once again, a number of significant parallels can be drawn between this theory and the concept of nongenetic information. Notably, these theories are both formulated in the context of evolution. In this review we aim to evaluate the role that nongenetic information, as we understand it in the modern day, plays in evolution.

**Properties of nongenetic information**

As discussed in previous sections of this review, it is widely accepted that nongenetic information plays a crucial role in the development of an organism's phenotype, which in turn determines how well that organism will fit its environment. It follows that nongenetic information may therefore influence phenotypic variation, alluding to a role for this information in evolution. Yet, the contribution of nongenetic information to evolutionary processes remains poorly understood. In order to fully appreciate the role that this information may play, it is first necessary to discuss the properties of the carriers which encode this information (Figure 2(B)). Ultimately, it is these properties which determine the possibilities and limitations of information encoded in nongenetic carriers.

Unlike genetic information encoded in DNA, which is replicated and equally partitioned during mitosis, there is often no requirement for this with regards to nongenetic information carriers and often these components are asymmetrically segregated (reviewed in (Shlyakhtina et al. 2019)) (Figure 4(C)). Thus, variation between cells can be produced across just one generation, which can lead to significant phenotypic variation.
within a population. Importantly, this can be the result of active or stochastic processes. Analogous to the concept of genetic drift in evolutionary biology, stochastic asymmetric segregation of nongenetic information carriers could alter the frequency of particular traits/phenotypes within a population, independently of any form of selection. Interestingly, one could assign this to a process of 'nongenetic drift', a potential mechanism of evolutionary change distinct from those pertaining to natural selection. Since this is viewed at the level of single cells, it follows that this process would be primarily implicated in populations of unicellular organisms or within populations of single cells. This highlights a significant difference between the evolution of unicellular and multicellular organisms. Processes such as asymmetric segregation have the potential to enable dramatic phenotypic shifts in unicellular organisms, potentially facilitating rapid adaptation to novel environments.

Interestingly, when conceptualized, it would seem that carriers of nongenetic information are often major nodes within signaling pathways which transduce signals originating from environmental stimuli (reviewed in (Arzate-Mejia et al. 2011)). Modifications of, and thus encoding of information into, nongenetic information carriers can therefore occur as a downstream result of environmental changes, signifying an ability of these carriers to store information originating from external stimuli. The transmission and interpretation of this information could subsequently enable changes in cellular phenotype to be matched to these environmental changes, unlike mutations which at first are random with respect to the environment. This positions carriers of nongenetic information at a crucial intersection between the environment and the phenotype, and has significant implications for evolution. If, as discussed above, evolution is viewed as a change in characteristics over time, and the changes directed by nongenetic information can be informed by and matched to environmental stimuli, it follows that this evolutionary process may represent a much more directed and efficient method of adaptation, which remains reversible and potentially short-term. Additionally, if these phenotypic changes are later encoded genetically (as will be discussed later in the review), this provides a mechanism for long term evolutionary change informed by the environment itself, which is distinct from the chance mutational change commonly referred to in the modern synthesis. Nevertheless, this does not negate the possibility that nongenetic information carriers can encode and transduce information originating from sources independent of environmental stimuli.

As opposed to genetic information which is solely encoded in DNA, there are numerous potential carriers of nongenetic information contained within a cell which all vary in their specific properties, including their potential modifications and conformation changes (Figure 2(A)). Importantly, this flexibility enables nongenetic information to be encoded in many diverse ways, and subsequently interpreted by or transmitted to a vast range of additional molecules. Further to this, changes to nongenetic carriers which enable the encoding of information tend to be readily reversible, either via active processes, such as demethylation of DNA, or alternatively via degradation or loss of the molecule by, for instance, the process of asymmetric segregation (Ramchandani et al. 1999; Shlyakhtina et al. 2019). In contrast, changes to DNA which result in altered genetic information are rarely reversible, mainly attributed to the superior stability of this molecule. This flexibility and reversibility is thought to be particularly advantageous when organisms experience a rapidly changing or fluctuating environment. In these conditions, it is believed that reliance on phenotypic change through alterations of genetic information alone would prevent an organism from reaching fitness peaks within the short time scales presented. For example, consider an organism living in an environment in which conditions fluctuate between two alternative states, A and B. The conditions change from A to B and a chance mutation confers an adaptive advantage to an organism in this environment. However, a short period of time later the conditions will change back to state A, and this mutation may no longer provide any fitness benefit to the organism, or may even be deleterious. Since this phenotype is encoded by information stored in DNA, it is not possible to simply revert back to the original phenotype and the organism must wait for further mutations in order to move to a fitness peak matched to these conditions. Consequently, DNA is more suited to long-term storage of information, which enables faithful inheritance over many generations when organisms experience stable environments. Conversely, phenotypes encoded by information stored in nongenetic carriers provide an advantage in unstable or fluctuating environments, offering a more short-term and flexible method of storage (Figure 3(B)).

Importantly, when considering the role of nongenetic information in evolution, perhaps the most significant property of the carriers involved is the time scales upon which they act. Specifically, the information encoded by nongenetic carriers can change on a much shorter time scale when compared to genetic information. This confers significant evolutionary power when
organisms encounter fluctuating or rapidly changing environments, similar to the properties discussed above, since it can potentially facilitate rapid phenotypic change unmatched in speed by that conferred via genetic change. This can be exemplified by the development of a multicellular organism. As is widely accepted, all cells present in an organism are largely genetically identical and yet significant heterogeneity is apparent when comparing different cell types. Considering the large extent of genetic homogeneity, it can be inferred that changes in nongenetic information underlie this variation. Importantly, this variation is produced in a relatively short space of time - specifically, the time it takes for the development of a multicellular organism, which highlights the pace at which changes to nongenetic information can produce dramatic shifts in phenotype.

Overall, a number of key differences between nongenetic and genetic information carriers can be identified (Figure 2(B)). These differences have important implications for the role of nongenetic information in evolution which will be discussed in more detail below. In certain situations, it can be proposed that this method of information storage confers significant evolutionary power to organisms; the ability to integrate environmental information, and the speed at which changes can occur enables an organism to potentially respond much more effectively to its environment and keep responding and changing, if required. Importantly, this can be attributed to the properties of the information carriers themselves.

Box 1. The history of nongenetic information carriers

Often when considering the role or function of a biological process, a valuable insight is gained from an understanding of its history and the conservation between, and also within, phylogenetic groups. This is an approach we will now take with our focus on nongenetic information carriers. However, as a consequence of our broad definition of nongenetic information, which in turn encompasses a wide range of potential carriers, our discussion will be limited to a key subset of these to illustrate a number of key concepts.

RNA
Possibly one of the most ancient nongenetic information carriers is RNA, with a considerable wealth of evidence supporting the hypothesis that this form of information storage even predates DNA. This is the central concept of the RNA world hypothesis, which posits that at the beginning of life on Earth, RNA functioned as the primary information carrier (Higgs and Lehman 2015). Many of the ideas surrounding this hypothesis were drawn from considering the basic requirements for life, which all center around information. In order for life to emerge and persist there must be mechanisms in place for the storage, replication, modification and conversion of information (into chemical reactions). Unlike polypeptides, nucleotides fulfill these criteria due to their ability to self-replicate (Lincoln and Joyce 2009; Attwater et al. 2013; Robertson and Joyce 2014). Indeed, the core basis of the RNA world hypothesis is rooted in the unique capabilities of RNA molecules, specifically their ability to store information in addition to acting as catalysts (Higgs and Lehman 2015). Notably, significant versatility has been demonstrated by RNA molecules, reinforcing the idea that they could have supported the core of a relatively complex life system. Moreover, chemical differences between RNA and DNA, in particular the demonstration that in modern cells deoxyribose is synthesized from ribose, further supports the idea that RNA functioned as the key information carrier prior to the emergence of DNA (Lazcano et al. 1988). Key experimental evidence to support and enhance these ideas has been provided by numerous studies since the initial formulation of the hypothesis. Classical experiments, as summarized in Higgs and Lehman 2015, established the field of in-vitro evolution and began to demonstrate the capabilities of RNA in this setting (Higgs and Lehman 2015). Further exploration of these principles led to landmark papers detailing the ability of RNA to self-replicate, strengthening the claim that this mechanism could have played a crucial role in the development of early life on Earth (Lincoln and Joyce 2009; Attwater et al. 2013; Robertson and Joyce 2014). Nevertheless, it is important to emphasize that the modern-day experiments and observations used here as evidence clearly cannot be claimed as proof of this hypothesis, instead, they can only indicate that the composition and processes that may have occurred in this RNA world are plausible. Importantly, it will never be possible to truly confirm the RNA world hypothesis, only gather evidence to support or refute its claims.

Despite the apparent suitability of RNA as an information carrier, at some point in the history of early life on Earth there emerged a novel polynucleotide, DNA. Subsequently, DNA claimed a key role as a hereditary information carrier offering improved structural stability, thereby enhancing long term storage and propagation of information. The majority of lifeforms are now DNA-based, in that they use DNA as their primary hereditary material. The shift to DNA is argued to have significantly contributed to the increase in organism complexity and transition to multicellularity later in history. Yet, there are still RNA-based lifeforms present today, namely RNA viruses if one can conclude that viruses are indeed living organisms). Interestingly, these viruses generally show an increased rate of evolution, attributed to the lack of proofreading associated with RNA polymerases (Holland et al. 1982). Regardless of the debate over virus classification, many still expect that DNA-free organisms separate from viruses will be found, and that the lack of evidence is solely due to a lack of exploration surrounding this possibility (Hyoshi et al. 2011). Nonetheless, a total shift out of the RNA world was never fully completed, as evidenced by the findings that RNA continues to carry out a role in information storage, which is often considered as a molecular fossil of the ancient world (Zhang and Chen 2019). It is important to note that RNA was in no way found redundant in DNA-based organisms. In evolutionary terms, it seems likely that the retention of RNA and its ability to store information is explained by its unique combination of features. Perhaps most notable is its ability to be modulated independently of any alteration to the genetic sequence, and the time scale on which these changes can appear and be reversed (reviewed in (Roundtree et al. 2017; Schaefer et al. 2017)). It must be emphasized that the evolution of components discussed in this review was often occurring concurrently, and so the evolutionary history of one can frequently be understood by considering possible co-evolutionary interactions. For instance, the potential shift from the RNA world to DNA-based organisms is thought to have facilitated an increase in organism complexity due to the ability to faithfully inherit (almost) error-free information over many generations. However, this was accompanied by an associated cost, in that the static nature of DNA means that a cell cannot produce immediate phenotypic changes in response to environmental stimuli through a change genetic sequence alone. Therefore, the properties of RNA discussed above may have enabled it to fulfill this critical role and co-evolve alongside the emergence of DNA to occupy this functional niche within the cell. In this way, it is likely that a significant evolutionary advantage was granted to organisms employing this strategy involving the storage of nongenetic information in RNA.

DNA methylation
As mentioned above, a key strategy often employed to gain an understanding surrounding the history of biological components
or processes centers around their comparison in different species of varying phylogenetic distances. Limiting this study to well-known model organisms is often insufficient for this line of research, and the use of non-model organisms is crucial in furthering our knowledge in these areas. This approach is discussed in a recent review which utilizes eukaryotic algae known as diatoms to infer the evolutionary histories of particular nongenetic information carriers (Rastogi et al. 2015). Phylogenetically, it is thought that diatoms diverged early from the eukaryotic lineage, providing a valuable opportunity to obtain information about the evolutionary history and origins of eukaryotic cellular components and processes. Interestingly, these organisms show rapid adaptation when subject to fluctuating environmental conditions, such as temperature and nutrient availability. This is mainly attributed to highly conserved epigenetic/nongenetic machinery found within these species, including small RNAs, histone modifying enzymes and, notably, DNA methylation. There is evidence of the latter in wide-ranging taxonomic groups, including plants, animals and fungi, however the components and mechanisms associated with this nongenetic information carrier are known to vary between species (Feng et al. 2010; Zemach et al. 2010; Bewick et al. 2019). Specifically, methylation of carbon-5 of cytosine, the most common modified base in eukaryotic genomes, is produced by a conserved group of enzymes in bacteria, archaea and eukaryotes (Goll and Bestor 2005; Zemach and Zilberman 2010). The original function of DNA methylation has been the subject of speculative debate for a number of years. Most prevalent is the argument hypothesizing that DNA methylation arose in early life-forms as a mechanism to silence transposable elements, thereby protecting genomic integrity. (Yoder et al. 1997; Slotkin and Martienssen 2007; Mirouze and Vitte 2014; Sigman and Slotkin 2016) and that further throughout its evolutionary history it was co-opted to carry out additional functions including the modulation of gene expression. For instance, it had been previously shown that a species of diatom, P. tricornutum, represses certain transposable elements through methylation at their genomic location (Maumus et al. 2009). Upon demethylation, via stimuli such as nitrate starvation, increased expression of these elements can be observed (Figure 2(C)). Indeed, it is hypothesized that this may function to provide genetic variation and innovation as a substrate for selection to act upon when encountering a novel environment. Moreover, the comparison of an, albeit limited, range of eukaryotic species returns an apparent correlation between levels of cytosine methylation and transposable element content (Rastogi et al. 2015). Further analysis of the methylome and methylation machinery present in P. tricornutum supports an ancient origin for this pathway. Interestingly, variations have been found in the enzymes which function in methylation pathways between eukaryotic lineages. From this, it can be inferred that evolutionary loss has occurred in certain lineages, however the commonalities and patterns this data presents provide evidence to suggest ancient origins for this information carrier.

Whilst this data can contribute to the understanding of evolutionary histories, it is important to proceed with caution when drawing conclusions. Comparisons such as these are empirically limited to the number of species which have had the relevant analysis carried out. Comparisons are strengthened by inclusion of phylogenetically distant species, yet the need for equivalent analysis to those found in eukaryotes. However, the huge variation between organisms may result in inaccurate conclusions if similar components or processes are present but just in unrecognizable forms. By way of illustration, a number of studies have previously claimed the absence of methylation in species such as C. elegans and D. melanogaster, primarily based on the findings that these organisms lack homologs of certain human DNA methyltransferases (Lyko and Malecszka 2011). However, it is now thought that DNA methylation may be present, albeit via an alternative pathway (Dunwell and Pfeifer 2015; Greer et al. 2015). Overall, these caveats highlight just some of the difficulties associated with these approaches, and care must be taken when drawing conclusions.

Histones
Utilizing a similar approach to the one detailed above, insight has been obtained into the evolutionary history of another nongenetic information carrier, histones. Structural and functional conservation of these proteins has been shown throughout the eukaryotic lineage, where they facilitate packaging of the DNA into nucleosomes, the basic unit of chromatin, alongside their function in the regulation of gene expression. Historically, it was inferred from their role in the packaging of DNA that histones were restricted to eukaryotic cells, since these are unique in their requirement to tightly pack genetic information into a membrane-bound nucleus. Despite this, data from a phylogenetic group distinct from eukaryotes, the archaea, indicates that certain species of these organisms also form nucleosomes made up of homologs of histones H3 and H4 (Pereira et al. 1997; Pereira and Reeve 1998), albeit with slight structural variations compared to those found in eukaryotes. During that last decade, the first nucleosome occupancy map for an archaeal genome was published, specifically, the species Haloferax volcanii (Ammar et al. 2012). Importantly, striking similarities were found when compared to eukaryotic chromatin, including nucleosome depletion localized at transcriptional start sites, indicating that this feature of chromatin is conserved between these distant groups. Furthermore, a correlation was also identified between nucleosome occupancy and gene expression. It has been argued that these results provide evidence to support the idea that histone proteins not only evolved before the divergence of eukaryotes and archaea, and are therefore evolutionarily ancient, but that their original function was in fact the regulation of gene expression. The latter conclusion derives from the realization that histones likely evolved prior to the existence of membrane bound DNA, since archaea lack a nucleus, and therefore do not require the same level of compaction exhibited in eukaryotes. Interestingly, this suggests that histones evolved primarily as a nongenetic information carrier and were later co-opted in the eukaryotic lineage to assist in the move to nucleus-bound genetic information. The initial emergence of histone-modulated gene expression is perhaps explained by the heightened need for short-term/immediate change in response to environmental stimuli which could not be provided by the carrier of genetic information, DNA. If, as hypothesized above, early lifeforms shifted from the use of RNA to DNA as the primary information carrier, this likely accompanied a reduced level of flexibility and potential for immediate adaptation to new environments. It follows that a mechanism offering modulation of gene expression, which is immediate and reversible, provides a significant evolutionary advantage over the reliance on chance mutational change in the genetic sequence itself. Herein lies one of the core concepts which could, in theory, provide an explanation for the evolution of many of the nongenetic information carriers we discuss in the context of this review.

How can nongenetic information contribute to evolution
One of the key arguments proposed in support of the Extended Evolutionary Synthesis centers around the influence of the environment on a organisms’ characteristics and the role of epigenetic mechanisms in adaptation and evolution. It has since been proposed by a number of authors that nongenetic information can have a significant contribution to evolutionary processes, with an ever-growing literature of evidence (Richards et al. 2010; Holeski et al. 2012; Liebl et al. 2013; Skinner et al. 2014; Sarkies 2020). Significantly, a role for nongenetic information has been implicated in key evolutionary transitions, including the major transition from uni- to multi-cellularity (Danchin et al. 2011). It is thought that nongenetic information carriers, initially functioning in cell plasticity and protection against
mobile elements, were co-opted for a role in cell differentiation enabling specialization of cells for diverse functions, fueling the progression and leap in complexity of these multicellular organisms. Nevertheless, the contribution of nongenetic information to more general evolutionary processes cannot be ignored, in particular through its ability to inform the phenotype and therefore influence a variety of phenomena, notably, nongenetic heterogeneity and phenotypic plasticity (see subsequent sections).

**Nongenetic heterogeneity**

Evolution via natural selection requires variation to act upon, commonly viewed in the sense of phenotypic variation which confers differential fitness between organisms resulting from the way different phenotypes interact with the environment they experience. This phenotypic variation is often assumed to be the product of underlying genetic variation within a population. Yet, it can be inferred that since nongenetic information can inform phenotype, then phenotypic variation could also result from nongenetic heterogeneity, thus implicating this in the process of evolution by natural selection (Holeski et al. 2012; Liebl et al. 2013; Skinner et al. 2014). For instance, in unpredictable environments, the generation of phenotypic heterogeneity can represent an advantageous strategy to increase the evolutionary potential of a population and ensure survival at the population level. This is known as bet hedging; the generation of multiple distinct phenotypes within a population with the aim that at least one will be well suited to the new or soon-to-be-encountered environment, ensuring survival of the population (O’Dea et al. 2016). Notably, it has been proposed that heritable bet hedging could facilitate movement between fitness peaks in the adaptive landscape (Pal and Miklos 1999). However, evidence of this strategy in multicellular organisms remains to be demonstrated. Nevertheless, bet-hedging is well documented in bacteria, which offers important insights into this mechanism and how it could potentially function in more complex multicellular organisms. In clonal populations of bacteria, it has been observed in numerous different species that often there exists a small subpopulation of cells with a slow or arrested growth state (Balaban et al. 2004). Notably, upon treatment with antibiotics, these cells endure and persist, enabling the population to survive the challenge. Importantly, this has been shown to occur without genetic changes and therefore must be solely underpinned by nongenetic information. Following treatment, this subpopulation of cells is able to spontaneously switch their growth state and produce a new population with the same composition and dynamics as the original starting population, including cells which are susceptible to the antibiotic. Computational modeling suggests that this stochastic switching occurs at steady state and resistance is conferred by preexisting heterogeneity (Kussell et al. 2005). Historically, it was thought that this strategy had been selected for since it provides a population fitness advantage when faced with environmental stress (Balaban et al. 2004). However, a recent study suggests it can benefit cells even when the environment remains constant (Levy 2016). Intriguingly, population-level fitness is seen to be increased with greater variation in growth in steady state conditions. This data calls into question the evolutionary explanation for why populations may contain slow growing cells, and suggests that these subpopulations may be simply a result of selection for higher variability in growth due to the fitness advantage it offers, which in this case can be provided by nongenetic heterogeneity.

Nongenetic heterogeneity is also thought to contribute to the evolution of organisms in the face of dramatic changes to the population with which they belong. In the wild, populations occasionally experience extreme reductions in size, for example, following events which cause the death of a majority, or through colonization of a new environment by a small founder group. In such events, there is commonly a dramatic reduction in genetic diversity associated with these situations, which results in phenomena such as genetic bottlenecks and the founder effect. The way in which many populations facing these genetic barriers are able to overcome them is viewed as a genetic paradox and has intrigued scientists for many years (Allendorf and Lundquist 2003). It has now been hypothesized that nongenetic information, specifically nongenetic heterogeneity, could offer important evolutionary potential in these situations (Pérez et al. 2006). In a similar way to bet-hedging, nongenetic heterogeneity could provide the phenotypic variation necessary to overcome these barriers when sufficient genetic diversity is not available. Notably, it has been shown that variation in certain nongenetic information carriers such as DNA methylation can be independent of, and even exceed, the genetic variation available in a system, supporting the hypothesis that even in situations of reduced genetic diversity nongenetic heterogeneity could provide alternative paths for adaptation and evolution (Herrera and Bazaga 2011; Richards et al. 2012; Schrey et al. 2012).
Beyond this explanatory hypothesis, there is some evidence to support the role of nongenetic information in overcoming this genetic paradox. A number of these studies utilize Darwin’s finches, historically renowned for their ability to respond to environmental change and generate phenotypic variation (Ranganath 2018). Comparative analysis of DNA methylation between species of finch using a variety of approaches have given a number of important insights. Notably, a significant correlation between methylation differences and phylogenetic distance suggests that these changes in nongenetic information accumulate over evolutionary relevant periods of time (Skinner et al. 2014). In comparison, no correlation was found between genetic changes and phylogenetic distance in the same study group. Similarly, significant differences in methylation were identified between populations of the same species found in different environments, where there were no significant genetic differences (McNew et al. 2017). Importantly, this is consistent with the hypothesis that nongenetic heterogeneity can contribute to rapid adaptation when faced with changing environments. However, these studies measure genetic variation via copy number variation analysis only (Skinner et al. 2014; McNew et al. 2017), which represents only a fraction of the potential genetic variation, therefore limiting the conclusions which can be drawn.

Interestingly, instances in which species are formally introduced into a new environment offer a unique opportunity to monitor the evolutionary processes occurring in these situations, often analogous to populations experiencing bottlenecks or the founder effect. Along these lines, a recent study explored DNA methylation patterns in a population of house sparrows undergoing expansion in a relatively new environment in Kenya (Liebl et al. 2013). Interestingly, increased heterogeneity in methylation levels were found in these birds, and this diversity negatively correlated with genetic diversity. This supports the idea that nongenetic variation could provide a compensatory mechanism in the presence of reduced genetic diversity, as is experienced by founder populations or following genetic bottlenecks. However, the results of these studies comprise purely correlations which cannot be used to infer causation, and so caution must be taken when conclusions are to be drawn.

**Phenotypic plasticity**

As explained above, understanding the properties of nongenetic information carriers can provide valuable insights into the potential roles they play in evolutionary processes. The ability of carriers to respond to environmental changes, in addition to the influence of nongenetic information on the phenotype places this information at the center of a phenomenon known as phenotypic plasticity. This describes the ability of an organism to alter their phenotype in response to environmental stimuli which occurs in the absence of any genetic change, inferring that changes exclusively in nongenetic information underlie this process (Schlichting 1986) (Figure 3(B)). Phenotypic plasticity can be identified at a number of different levels, including changes to an organism’s developmental program, morphology or behavior, which are associated with slightly varying properties, for instance the speed of response and the ability of the response to be reversed. The plasticity of a trait is classically represented as the slope of a reaction norm, derived from plotting environment versus phenotype. In essence, this reaction norm denotes the extent of phenotypic change produced by changes in nongenetic information in response to different environmental conditions.

A common example of phenotypic plasticity is found in the morphology of the feeding apparatus in response to the food available during a metazoan organism’s development (Wimberger 1992; Walker 1997; Wintzer and Motta 2005; Sommer et al. 2017). This is exemplified by the European eel, whose diet-induced plasticity in head morphology is thought to enable individuals to specialize in feeding upon a certain prey, and may reduce competition within the population (De Meyer et al. 2016). In situations akin to this, or in environments which fluctuate or change unpredictably, it is clear that the ability to express phenotypic plasticity in certain traits will provide an adaptive advantage to organisms. It is now understood that the extent to which specific traits show plasticity can be genetically encoded and therefore acted on by natural selection if advantageous. However, the evolutionary potential of the phenotypes themselves which are produced by plastic responses represents a long running debate. Historically, scientists have recognized its importance in evolutionary processes and, more recently, the Extended Evolutionary Synthesis (EES) has included phenotypic plasticity as a possible initial step in adaptive evolution (Baldwin 1896; Waddington 1942a; West-Eberhard 2005; Danchin et al. 2011). An early advocate of the ideas now attributed to the EES, Karl Popper, explained the logic behind a role for plasticity in evolution and reasoned that organisms will not wait for chance mutations following environmental changes, they will instead actively adjust their phenotype through alterations in gene expression (Jablonka 2017). Others share his theoretical standpoint,
supporting the idea that nongenetic information, in particular through its role in phenotypic plasticity, could provide a mechanism for organisms to overcome barriers in fitness landscapes by increasing the dimensions of phenotypic space encoded in the genome (Shorter and Lindquist 2005; Badyaev 2014) (Figure 3(A)). Furthermore, phenotypic plasticity is by no means independent to nongenetic heterogeneity described previously. In fact, it is plausible to predict that nongenetic heterogeneity can arise from phenotypic plasticity in multiple ways, and vice versa, in addition to deriving from stochastic processes. Firstly, the plasticity of certain traits may vary between organisms, either via genetic or nongenetic variation, and an environmental change may produce different responses in the organisms resulting in nongenetic heterogeneity within the
population. Additionally, different organisms will often experience different environmental stimuli, or varying strengths of the same stimulus, which will induce distinct plastic responses and subsequent heterogeneous phenotypic alterations. Overall, it becomes clear that phenotypic plasticity has significant potential to contribute to the evolutionary trajectory of a population.

However, to date we still lack an understanding of how many of the plastic responses observed come to be, and precisely how nongenetic and genetic factors contribute, potentially together, in response to environmental changes (O’Dea et al. 2016). It is widely appreciated that it remains difficult to directly demonstrate the adaptive potential of phenotypic plasticity (Merila and Hendry 2014). Nevertheless, absence of evidence cannot be taken as evidence of absence. Indeed, it has even been suggested that non-adaptive plasticity can still contribute to evolutionary processes, by moving populations farther from fitness peaks and in turn strengthening directional selection which acts on genetic variation present between organisms, yet the evidence for this remains limited (Ghalambor et al. 2007; Ghalambor et al. 2015; Sentis et al. 2018).

In order to overcome the difficulties encountered when gathering evidence in the field, a number of studies have approached this question using mathematical modeling. For instance, Mostowy et al. model the effect of nongenetic information and phenotypic switching on antagonistic relationships, or co-evolution (Mostowy et al. 2012). Often when these relationships are modeled without considering nongenetic information as a factor, oscillatory dynamics are generated which cannot be observed in data gathered from populations in nature. Interestingly, the inclusion of nongenetic information as a variable into the model eliminates these oscillations and better reflects data obtained from the field, suggesting that nongenetic information does indeed play an important role in the evolution of these relationships.

When considering the role of nongenetic information in these processes, a key aspect to highlight is its ability to decouple phenotypic change of an organism from its genotype (Bonduriansky and Day 2009). This potentially enables the phenotypic landscape to be altered in new ways, independent of genetic information. This decoupling may also allow organisms to preserve genetic variation when subjected to rapid environmental change. For instance, drastic environmental change would normally reduce genetic variation and impact on future evolutionary potential. However, if nongenetic information can still provide phenotypic variation independently of the genotype, it could reduce the loss in genetic diversity whilst still ensuring survival and propagation (O’Dea et al. 2016). Hence, it is argued by many that nongenetic information is particularly important when facing rapid environmental change, when genetic variation cannot keep pace. This concept is termed epigenetic buffering and can potentially explain how a population, upon encountering drastic environmental change, can avoid extinction in the short term, which increases the time for genetic change to occur, preserves genetic diversity and promotes future genetic variation (O’Dea et al. 2016).

The interaction between nongenetic and genetic elements

Despite the emphasis on the decoupling of genetic and nongenetic components, it is also important to consider that these elements co-evolved and likely benefited from a certain degree of cooperation which enabled exploitation of their individual properties.

A key example of a case in which nongenetic and genetic information carriers interact, with evolutionary consequences, is the masking and subsequent expression of cryptic genetic variation (CGV) by nongenetic information carriers (Paaby and Rockman 2014). CGV is genetic variation, for instance, changes in the DNA sequence, which has no effect on the phenotype under normal environmental conditions. Notably, this enables this variation to evolve neutrally in the absence of selective pressures. Upon environmental change, this variation may be released and hence be expressed, potentially influencing phenotype. This ability to store evolutionary novelty silently and then release it during periods of environmental change is thought to represent an advantageous evolutionary strategy. This strategy reflects historical ideas, in particular those proposed by Waddington in his theory of canalization (Waddington 1942a). He hypothesized at the time that development is robust and is able to maintain a stable phenotype despite accumulation of slight genetic variation (canalization), however in certain conditions this hidden variation can be revealed (decanalization), allowing novel and potentially adaptive phenotypes to be expressed (Waddington 1942a).

There are a number of mechanisms identified by which CGV can be released, mediated by different carriers of nongenetic information (referred to here as capacitors); however, the evolutionary implications are comparable between all. A key example of a capacitor of CGV release is the prion [PSI+] (Figure 2(C)). Prions are self-propagating protein isoforms, thought to act as nongenetic information carriers which can be inherited.
across cell divisions via the cytoplasm (Shorter and Lindquist 2005). Prions are often found to be pathogenic, however there is evidence to support their function in phenotypic variation and evolution of organisms. [PSI+] is found in yeast, is the result of a conformational change and subsequent aggregation of the protein Sup35. Sup35 is identified as a translation termination factor, however its function is impaired upon switching to the [PSI+] form. Interestingly, phylogenetic analysis indicates that this ability to switch to the [PSI+] state has been conserved for over one hundred million years in the yeast lineage (Harrison et al. 2007).

Importantly, it has been shown that the [PSI+] state can result in exposure of CGV through the impairment of translation termination, resulting in ribosomal read-through and extension of the translated sequence at the 3’ end (True et al. 2004). Previously untranslated regions, a source of potential genetic variation, will now be incorporated into the product molecule. Interestingly, the phenotypes produced from the release of this CGV can be advantageous and promote increased growth rates under stress (True and Lindquist 2000). Despite this, the [PSI+] state reduces translation termination fidelity and is likely maladaptive in the long term. However, there is evidence to suggest that the potentially adaptive phenotypes produced by the [PSI+] state can be rendered independent of [PSI+] and persist in its absence. True et al. show through outcrossing experiments that the variation revealed by [PSI+] was fixed and phenotypically present in subsequent generations after this prion was removed from the system (True et al. 2004). It is thought that this is a result of alterations in genetic information, specifically in stop codons of the reading frames involved, yet the mechanism for this remains unknown. This ability to assimilate beneficial phenotypes is proposed to facilitate the evolution of yeast populations. [PSI+] may represent a short-term advantage for survival in novel conditions, increasing the chance that these new phenotypes will be fixed by genetic changes. Interestingly, yeast cells have been shown to switch between the prion and non-prion states more rapidly when stressed, providing additional support to the theory that this mechanism provides an adaptive advantage in novel environments (Tyedmers et al. 2008).

In evolutionary terms, the [PSI+] mechanism essentially enables sampling of regions which have not been subject to selective pressures, which can then be tested in the yeast cells. Specifically, increased switching to the [PSI+] state in response to stress, or stochastically, confers the potential to access hidden variation which could provide an adaptive advantage, facilitating survival and propagation in the face of stress or novel environments. This increases the time available for adaptive traits to be assimilated and increase in frequency within the population. In this line of thought, we can hypothesize that errors are tolerated in biological systems as CGV and it is plausible that this mechanism is not restricted to yeast. Importantly, CGV enables many polymorphisms to accumulate without any immediate phenotypic change. Subsequent release by the [PSI+] state can thus result in dramatic phenotypic shifts which is thought to enable potentially significant evolutionary jumps and rapid evolution of complex traits.

The influence that the properties of nongenetic carriers have on evolutionary processes is exemplified by the action of prions. Prions can generate novel phenotypes without commitment, unlike genetic mutations. The prion state can be readily lost from cells - for instance, through asymmetric segregation - resetting the cells back to the non-prion state and eliminating a potential mal-adaptive or non-adaptive phenotype. Overall, it is clear that the impact of nongenetic information, through the exposure of CGV, is significant and can provide evolutionary power in the face of stress and novel environments. States induced upon prion formation, such as [PSI+] provide mechanisms to couple genetic diversity and environmental variation to drive evolutionary change.

Neutral and maladaptive contributions to evolution

When considering the role of a process or component in evolution, there is a tendency to focus solely on how it may grant an adaptive advantage and provide changes to organisms which enable them to move to fitness peaks, ultimately becoming better suited to their environment. However, there are many other ways in which a contribution to evolution can be made, which do not necessarily confer an adaptive advantage, since evolution is simply ‘change over time’ with no requirement for the change to be beneficial.

An example of this is illustrated by the process of nongenetic drift, proposed in an earlier section of this review. Nongenetic drift would result in a change in frequency of certain phenotypes due to shifts in nongenetic information, independent of any selective pressures. Since these changes are stochastic and due to chance only, the change in phenotype frequency within a population does not necessarily reflect an adaptive shift and subsequent increase in population fitness; instead, it likely acts neutrally with regards to
fitness. Nevertheless, this process still results in a change in characteristics of organisms over time and therefore can be considered as evolutionary change. This exemplifies the fact that not all evolutionary processes must confer an adaptive advantage, and offers an alternative mechanism by which nongenetic information can contribute to evolution.

Similarly, the role of nongenetic information in maladaptive changes must also be considered. For instance, there are documented cases in which plastic responses are inappropriate for the environment the organism experiences, which could arise due to the parental environment being a poor predictor of offspring environment (Langerhans and DeWitt 2002). Additionally, many examples of transgenerational epigenetic inheritance (discussed in detail in a later section) observed in an experimental setting, such as an abnormal diet or stress conditions, lead to poor health in the offspring which could be viewed as maladaptive (reviewed in (O’Dea et al. 2016)) (Veenendaal et al. 2013; Dias and Ressler 2014; Gapp et al. 2014). However, the presence of this response suggests that it provided a beneficial function to some degree in the life history of this species. It could be argued that this represents an ‘evolutionary trap’, where the stimulus used by an organism to assess the fitness of a trait becomes inappropriate or mismatched, and is subsequently maladaptive in a particular situation. Since these phenotypes are produced due to changes in nongenetic information, it has been described as an ‘epigenetic trap’ (O’Dea et al. 2016). Nevertheless, although some responses appear maladaptive, they may serve to increase variation which could confer evolutionary potential in the long term. Interestingly, it has been proposed that nonadaptive plasticity may in fact function to drive faster adaptation to environmental changes (Ghalambor et al. 2015). Theoretical models provide support for this idea, indicating that nonadaptive plasticity reduces population fitness, in turn strengthening directional selection due to the induced phenotypes being further from fitness optima. Ghalambor et al. show that the direction of changes in gene expression following a plastic response is, in general, opposite to the direction in which adaptive evolution proceeds (Ghalambor et al. 2015). These results appear to provide support for the theoretical models, yet many remain skeptical of these ideas and further evidence is required to fully understand the implications of this phenomenon.

In addition to playing a role in evolutionary processes itself, nongenetic information can also influence the overall rate of evolution. As discussed earlier, certain nongenetic carriers, including DNA methylation, function in the silencing of transposable elements (Slotkin and Martienssen 2007). The movement of these elements in the genome is thought to represent a source of significant genomic change and therefore has the potential to contribute significantly to the evolution of a species. It could be argued, therefore, that nongenetic carriers can prevent these events from occurring through their role in silencing, thereby reducing the rate of evolution.

Overall, it is clear that there are many mechanisms that contribute to the evolutionary process, even if this is not completely clear at first glance. It is important, therefore, to remain open to these alternative mechanisms in order to fully assess the role of nongenetic information in evolution.

The problem of inheritance

When considering evolution by natural selection (whilst also acknowledging that this is not the only mechanism to achieve evolutionary change), it is possible to identify three fundamental requirements necessary for a trait to be acted on by this mechanism; phenotypic variation, fitness differences and inheritance of phenotype by offspring. Nongenetic information can satisfy the first two criteria, by influencing an organism’s phenotype and producing phenotypic variation which confers variation in fitness, however the third is a controversial topic of debate. For unicellular organisms, inheritance of phenotype only requires transfer of information across one cell division to be inherited by daughter cells which makes identification and demonstration of nongenetic inheritance much simpler. Notably, a now classic example of inheritance at the structural/morphological level was first reported half-a-century ago in unicellular organisms, in pioneering work on cortical inheritance in the ciliate Paramecium (Beisson and Sonneborn 1965; Beisson 2008; Fields and Levin 2018). However, in the case of a large number of multicellular organisms, for information to be inherited it must be present in or transferred to the gametes and maintained throughout development to successfully recapitulate the parental phenotype. This process is commonly known as transgenerational epigenetic inheritance (TGEI) (Roemer et al. 1997). A key question when assessing the role of nongenetic information in evolution must be the extent to which it can be inherited and consequently involved in the process of natural selection.

It is now accepted by many that limiting the concept of heredity to solely the inheritance of information
through DNA is no longer accurate (Danchin et al. 2011). Many modern evolutionary frameworks support the inclusion of nongenetic inheritance, including the EES, however it has been difficult to confidently link nongenetic information to evolution since it is unclear as to what extent it is heritable. Interestingly, the debate surrounding nongenetic inheritance is not new. The idea that the environment can influence an organism’s phenotype and this can be inherited dates back to the theory of Inheritance of Acquired Characteristics, which proposed that traits acquired by parents were inherited by the offspring (Condorcet 1794; LeRoy 1802). As explained previously, this theory is commonly associated with Lamarck’s theory of evolution which throughout history has been discredited and met with significant resistance. This association may provide a partial explanation as to why the inheritance of nongenetic information is also met with resistance in the modern day, since it is often wrongly interpreted as a mechanism for the inheritance of acquired characteristics in the context put forward by Lamarck. Additionally, the narrative taught to many when first approaching the subject of evolution is that Lamarckian evolution, including the inheritance of acquired characteristics, opposes the ideas of Darwin which are widely accepted. However, it is seldom appreciated that Darwin in fact did not dispute Lamarck’s ideas and even went as far as to propose a mechanism to explain the inheritance of acquired characteristics, known as his theory of pangenesis. This theory explains the concept of gemmules, carriers of information in cells that may be subjected to influence by environmental stimuli and can be inherited by the offspring through the germline. The modern view of TGEI reflects the ideas posed in those theories. Interestingly, a number of parallels can be drawn, in particular between gemmules and nongenetic information carriers, highlighting that the ideas surrounding TGEI have been considered in various forms for many years, even preceding any knowledge of genetics and cellular biology.

Despite this, the heritability of nongenetic information remains a topic of debate and is disregarded by many. A significant contributing factor to this is the lack of evidence for the inheritance of nongenetic carriers, especially in organisms whose developmental program involves removal of many chromatin modifications acting as nongenetic carriers, such as DNA methylation, during waves of reprogramming (Miska and Ferguson-Smith 2016; Skvortsova et al. 2018). As mentioned above, this represents a key difference when considering inheritance across generations of uni- versus multicellular organisms; unicellular organisms such as yeast only require carriers to be passed across one cell division in order to be inherited, whereas carriers in sexually reproducing multicellular organisms must pass through the germline in addition to persisting through multiple stages of reprogramming during development.

Despite this, there is evidence in the literature for TGEI, mainly focused on either demonstration of the effects of TGEI in offspring or potential mechanisms through which TGEI could occur (reviewed in (Jablonka 2013; Burton and Metcalfe 2014; Heard and Martienssen 2014; Boskovic and Rando 2018)).

Studies providing evidence for TGEI indirectly by measuring molecular and phenotypic differences in the offspring are extensively reviewed in (Jablonka 2013; Burton and Metcalfe 2014). In particular, it has been recognized for many years that environmental effects can be passed to subsequent generations in plants (Johannes et al. 2008; Bonduriansky and Day 2009). On the other hand, there is currently no direct evidence for TGEI in humans, however it has been possible in a number of cases to utilize records of populations which faced a common and major environmental stress. This includes the Dutch Hunger Winter, which led to extreme malnourishment, and the extreme trauma caused by the Holocaust. A number of studies have assessed the phenotypic and molecular effects of these events on survivors and the following generations, resulting in interesting correlations being identified; however, one must be cautious when drawing conclusions from this data and some of these studies have been met with significant resistance (Heijmans et al. 2008; Veenendaal et al. 2013; Yehuda et al. 2016).

Additionally, there have been concurrent efforts to identify potential carriers of nongenetic information which can persist across generations. Due to reprogramming events involving the almost complete erasure of DNA methylation and histone modifications (without neglecting the fact that there are novel DNA modifications yet to be mapped across embryogenesis and likely additional modifications which remain undiscovered), the focus for potential candidates has shifted in recent years to other carriers such as RNA. Due to its role in chromatin remodeling and ability in turn to be modulated by chromatin structure, this places it as a potential key player in TGEI. Notably, accumulating evidence emerging from research in nematodes supports the role of small RNAs in TGEI. Indeed, it has been shown that heritable small RNA-based responses to stimuli can result in phenotypic changes detectable in the offspring for multiple generations (Fire et al. 1998; Rechavi et al. 2011; Ashe et al. 2012; Rechavi and Lev 2017; Houri-Zeevi et al. 2020). Strikingly, evidence
obtained from a number of model systems suggests that nongenetic inheritance often occurs through the paternal line, and it is now recognized that sperm contribute more than simply the paternal DNA (Immler 2018 and references therein). For instance, in mice, numerous nongenetic information carriers have been identified in sperm, including RNA, proteins and chromatin modifications, however recent interest has focused on sperm RNAs. Indeed, the development of high-throughput analysis techniques has revealed the diversity of sperm RNAs, such as miRNAs and tRNA-derived small RNAs, alluding to the possibility that these could provide a mechanistic explanation for nongenetic inheritance (Peng et al. 2012). Notably, it has been shown that the composition of sperm RNA is altered following stress or dietary restrictions, potentially inferring a change in the nongenetic information being inherited by the offspring (Gapp et al. 2014; Chen et al. 2016; Schuster et al. 2016; Sharma et al. 2016; Sarker et al. 2019). It has also been shown that sperm RNA from these mice can produce measurable effects in the offspring, and a recapitulation of the parental phenotype (Chen et al. 2016; Chen et al. 2016; Sarker et al. 2019) (Figure 2(C)). Yet, it is still unclear which subpopulations of sperm RNA are responsible and how this information is stored and read in order to produce an observable phenotype. One emerging area of research with the potential to shed light on this stems from the discovery that RNA molecules can be heavily modified (epi-transcriptome). Indeed, it is currently appreciated that there are many additional layers of complexity contributing to the potential levels of information storage attributed to RNA molecules (Schimmel 2018) thus fueling the idea that a ‘sperm RNA code’ might exist (Zhang et al. 2019). In that regard, it is thought that this could enable the encoding of information, via a diverse array of RNA types and their modifications, that can subsequently be inherited and decoded during offspring development (Zhang et al. 2018).

As a result of the limited understanding gained from experimental methods, alternative approaches have been taken to assess the extent of nongenetic inheritance and its potential role in evolution, in particular, mathematical modeling. It has been hypothesized that the inheritance of nongenetic information provides an explanation to the discrepancy between evolutionary models centered around genetic information and empirical data gathered from the field. Models of evolutionary dynamics which integrate both genetic and nongenetic inheritance have been shown to make different predictions to those which omit nongenetic inheritance (Day and Bonduriansky 2011; Geoghegan and Spencer 2012). Specifically, one model anticipates that the inclusion of nongenetic inheritance leads to a wider range of possible evolutionary outcomes, which is claimed by the authors to result from the unique properties held by nongenetic carriers (Day and Bonduriansky 2011). It is suggested that these properties, such as the ability to transmit traits influenced by the environment and the possibility for non-Mendelian transmission, enable a decoupling of phenotypic and genotypic change, in turn influencing evolutionary dynamics. Interestingly, some models have shown that nongenetic inheritance is particularly advantageous when organisms are experiencing environments which are prone to frequent random fluctuations (Lachmann and Jablonka 1996; Herman et al. 2014).

Box 2. Drawing parallels between nongenetic information, evolution and AI (an interesting analogy)

In order to fully appreciate the function of nongenetic information, it may be insightful to take a radically different approach and draw analogies with a concept far from the field of biology – Artificial Intelligence (AI). AI is a broad field, generally referring to computational systems displaying ‘intelligence’ which mimic cognitive functions akin to the human brain. In principle, this involves machines able to integrate information from their environment and use this to maximize their chance of achieving an optimal output (Hamet and Tremblay 2017). A basic principle when programming AI is to encode core generic instructions or rules which the system can then apply to reality in specific ways, given certain inputs or environmental cues. The alternative, encoding specialized hard instructions that disregard any external input when producing the output, puts limitations on the system’s potential. Interestingly, when considering the approaches employed for AI programming, it is possible to draw analogies with biological information storage. For instance, a program which returns a standard output in the form of a predetermined response regardless of any specific input or context could be compared to genetic information alone informing the phenotype. At a basic level, if genetic information, essentially the DNA sequence, is considered in isolation then the same output will be generated regardless of the environment or any external stimuli. In other words, a single genotype will map to a single phenotype. However, a program which is able to take different inputs and alter or customize the output according to this information, whilst still using core encoded information, could be analogous to the addition of nongenetic information to a biological system. Thus, it confers the ability to take information from other sources at the time and integrate this with pre-programmed information to produce an output which will be appropriate for the current conditions or needs.

It could be proposed, therefore, that the evolution of biological information storage has favored a strategy which provides a faithful means of information inheritance to encode the core instructions required to produce an organism, whilst maintaining additional levels of nongenetic information storage to integrate environmental information and influence how the core instructions are interpreted. It is likely that this represents a more space efficient approach which limits the amount of information required to be stored and faithfully replicated in DNA, whilst allowing flexibility and plasticity.

Interestingly, this strategy for programming AI aims to increase the ‘adaptability’ of the system, which reflects the aforementioned evidence suggesting that nongenetic information is instrumental in the adaptation of organisms to their environment.

In terms of adaptation and evolution, analogies can also be drawn with computational systems within the AI field. Machine learning is a subset of AI, bestowing systems the ability to learn from
experience in an independent manner (Deo 2015). In general, terms, the concept of learning involves processes which alter over time as a result of past experiences. In the machine learning approach known as “reinforcement learning”, systems produce an output, usually interpretation of a dataset, which they then receive feedback on which in turn tunes the model used to interpret the data. This aims to improve future and alternative interpretations of the data; thus, learning can occur. Nevertheless, these are simply optimizing systems based on data interpretation. It could be argued that actual learning requires the system to understand and remember the process of learning, whereas AI cannot fulfill this and is limited to the aspects of learning which enable optimization of an output over time. Interestingly, it has been proposed that parallels can be drawn with evolutionary systems (Watson and Szathmary 2016). In particular, reinforcement learning is thought to provide a direct analogy to evolution by natural selection. In evolutionary systems, feedback is provided through differential survival and reproductive success of an individual which displays a specific phenotype (the output). As explained above, this phenotypic output is produced via integration of genetic information and environmental stimuli by nongenetic mechanisms. Therefore, in the process of natural selection, feedback is given on the success of this integration by the fit of the produced phenotype to the given environment. There are many instances in which organisms adapt to novel environments much faster than would be expected, for which scientists seek explanations. It is thought that evolutionary systems enabling the type of learning described above could facilitate this. However, at this point it is important to note that neither evolutionary systems nor machine learning systems can ‘predict the future’ and anticipate challenges which have not yet been presented. Instead, these systems can generalize using their ‘model’ which has been molded by feedback from past experiences and outputs. Hence, the learning component of evolutionary systems does not aid rapid evolution in the sense of predicting the future, but may enable the utilization of previous selection outcomes to generalize and interpret the current, potentially novel, situation in a more effective way. Essentially, past experiences constrain the adaptive potential of organisms, since it is these experiences which determine the genetic and nongenetic information passed to the next generation and remain integral to the ‘model’ for integration and production of the phenotype. Interestingly, since it is possible to draw parallels between the algorithmic principles of evolutionary and machine learning systems, it follows that novel insights could be made by applying theories from one to the other. For instance, the application of evolutionary theory to machine learning has enabled the proposal of a new approach to the production of general AI. This involves the development of an AI Generating Algorithm (AI-GA), which takes the principles of evolution to generate computational ‘intelligence’ (Clune 2019). Placing the production of AI in the hands of computational systems which can, through machine learning, learn to complete this task may enable this to be achieved more efficiently and effectively. To date, the only known example of the generation of intelligent life is through the process of evolution on Earth. Hence, the authors reason that an approach centered around evolutionary principles is a clear choice to attempt this task.

A key argument for the rejection of nongenetic inheritance and its contribution to evolution is based on an understanding of the properties associated with nongenetic carriers, notably their reduced stability and increased plasticity, which means that their long-term implications in evolutionary processes are debatable. However, perhaps at this point it is important to reexamine the specific question being asked. When assessing how nongenetic information can influence evolution, it is possible to consider a situation in which nongenetic information, likely in combination with genetic information, can function in the short term and still produce long term effects to direct the course of evolution. To reiterate, perhaps it is naive to assume that nongenetic information and its carriers must be inherited and persist long term in order to participate in evolutionary processes. Specifically, one could propose a mechanism for nongenetic information to be transferred into genetic information encoded in DNA, providing a much more stable vector for inheritance and long-term propagation, circumventing the arguments posed above. This transfer of nongenetic information to DNA is at the basis of a process known as genetic assimilation. Interestingly, the general concept of genetic assimilation can be found in theories dating from many years ago. In 1942, Waddington published his theory of canalization to propose an alternative evolutionary mechanism to natural selection of chance mutations (Waddington 1942a). This originated from his dissatisfaction with natural selection, stating “It is doubtful, however, whether even the most statistically minded geneticists are entirely satisfied that nothing more is involved than the sorting out of random mutations by the natural selective filter.” (Waddington 1942a). He proposed that the study of development could aid the understanding of evolutionary processes which bear similarities to the inheritance of acquired characteristics. A key example he gives involves the callosities of an ostrich, similar to the thickening of human soles during embryogenesis. Instead of the widely accepted mechanism involving chance mutation and natural selection, he proposes that an environmental stimulus (in this case, friction) produces these features over generations and over time the stimulus is replaced by genetic code. Ultimately, the feature becomes ‘canalized’. Demonstration of his theory is provided through experiments on Drosophila embryos, in which an induced phenotype eventually becomes independent of the stimulus and so is argued to have become genetically encoded (Waddington 1942a).

Along the same lines is an earlier concept put forward by James Baldwin, now known as the Baldwin effect. This proposes that environmental change results in adaptive plastic responses which confer a survival advantage, allowing organisms to persist for longer, in turn providing more time for adaptive traits to arise from random mutation (Baldwin 1896). In other words, phenotypic plasticity influences survival in a novel environment, leading to an increased probability that adaptation can occur through the classical means of chance mutations and natural selection.
Both canalization and the Baldwin effect share the same core concept – the idea that changes in nongenetic information in response to stimuli can eventually result in genetically encoded traits, by serendipity or targeted mechanisms. However, the role of nongenetic information differs between the two theories. In the Baldwin effect, nongenetic information only plays a role initially in the plastic response and is assumed to be independent to any genetic changes which later occur – ‘serendipity’ (Baldwin 1896). Conversely, canalization suggests that nongenetic information directly informs the subsequent genetic alterations which enable the trait to become independent of the stimulus – ‘targeted’ (Waddington 1942a).

It is important to appreciate that these are not competing or mutually exclusive mechanisms. It seems that the Baldwin effect is more applicable to scenarios where plasticity is selected for and not costly to maintain. Equally, canalization appears more relevant in situations where plasticity is costly and the environment is relatively stable, meaning that transfer of the plastic response into genetic information is evolutionarily advantageous.

Both theories have contributed to the modern understanding of genetic assimilation, the process by which phenotypic variation produced in response to environmental stimuli becomes independent of the stimulus (Pigliucci et al. 2006). There are numerous examples of this mechanism, including the demonstration in yeast that phenotypes generated in the [PSI+] state can become assimilated through loss of the stop codon, as discussed previously (True et al. 2004). Moreover, it is thought that in environments that are relatively stable, e.g. an environment which produces reliable stimuli, it is likely that a plastic response will be assimilated and the reaction norm will be flattened since plasticity is often costly to maintain (Relyea 2002; Van Kleunen and Fischer 2005).

Interestingly, it has been suggested that phenotypic plasticity followed by genetic assimilation may provide an explanation for examples of rapid adaptation which have intrigued scientists for many years. In particular, it could represent a valuable route for adaptation and evolution of organisms encountering novel environments, such as invasive species (Pigliucci et al. 2006).

Despite these powerful predictions, the mechanism(s) underlying the process of genetic assimilation remain poorly understood. There is some evidence that nongenetic information can inform changes to the DNA sequence, such as deamination of methylated bases, however further research is required (Chahwan et al. 2010).

Overall, the above discussions center around the concept of nongenetic information informing or enabling a change in genetic information in order to play a role in evolution. While this appears logical, mainly due to the different properties associated with nongenetic and genetic carriers, it remains important to ask if there is a fundamental requirement for genetic change. If one accepts some level of nongenetic inheritance, it becomes feasible to suggest that nongenetic information can drive evolutionary change in absence of any change to DNA sequence. By way of explanation, we have described how nongenetic information can inform phenotypic variation which, if inherited, can result in an adaptive change in the frequency of characteristics over time (evolution). Ultimately, it must be acknowledged that information carriers possess different qualities and so genetically encoded adaptive changes will likely be selected for in stable environments; however, it is perhaps misleading to focus exclusively on how a genetic change can be brought about and reject any possibility of a long-term contribution from a process which does not involve this.

Rethinking nongenetic inheritance

It may be naïve to approach aspects of nongenetic information with a reliance on knowledge of genetic information, yet it may be equally dangerous to approach these as entirely independent concepts. However, research into nongenetic inheritance is often undertaken with a tendency to utilize what is understood about genetic inheritance and prematurely construct frameworks based on this. Importantly, from what is now known about the inheritance of nongenetic carriers, this may be preventing the development and formulation of new ideas surrounding this process. The way in which certain nongenetic carriers are inherited may be fundamentally different to DNA. In particular, nongenetic inheritance through gametes is now thought to be a process of reconstruction not replication, as is the case for DNA (Jablonka 2013). In that regard, it has been proposed that erasure of chromatin modifications is not complete during reprogramming, and some persist into the offspring with the potential to assist in the reconstruction of modifications which were removed (reviewed in (Margueron and Reinberg 2010)). Therefore, demonstration that a certain nongenetic carrier is not present in gametes or cells of an early embryo cannot be taken as evidence of a lack of inheritance.

Along those lines, within the scientific community, many are conditioned into considering inheritance only
in terms of replication, a result of DNA being characterized and understood before nongenetic carriers. In this way of thinking, the process of inheritance is reduced to a number of stages; replication across cell division in the parent, persistence in the gametes and early stages of development, and once again replication across cell divisions during growth of the offspring. However, in the case of nongenetic information, searching for evidence of inheritance by identifying a specific carrier in the germline or offspring (an approach taken by many) may be insufficient or even inappropriate. As mentioned previously, due to the read-write nature of nongenetic carriers, it is possible for information to be transferred to an alternative carrier for transmission through the germline into the offspring, and then transferred back to the original carrier type following completion of development (Figure 4(A)). It may even be misleading to assume that information will be transferred back to the original carrier in the offspring. This issue is amplified in the case of nongenetic carriers due to their diversity compared to DNA. In that sense, it becomes challenging to assess the inheritance of nongenetic information by simply demonstrating the presence of the carriers themselves. Perhaps more insight would be gained from demonstrating inheritance and persistence of parental phenotypes induced by environmental stimuli, encoded by the nongenetic information we seek to understand. Yet, it could be argued that even if information is inherited, the way in which it is interpreted to produce a phenotype may be different in the context of the offspring. Importantly, offspring will contain information, genetic and nongenetic, from both maternal and paternal sources and it is known that the interpretation of information can vary in the presence of, and upon integration with, other types/sources of information.

In summary, it becomes clear that assessing the passage of nongenetic information across generations is not simple, and requires careful consideration when designing experimental approaches to demonstrate it. Additionally, it may be required to contemplate the concept of information itself more thoroughly. One of the difficulties in monitoring the passage of information is that information is not a physical entity, it can be encoded by a carrier and interpreted by a system but never identified as a molecule itself (Figure 4(A)). Due to the dynamic nature of cellular systems, the carrier in which information is encoded and the state of the system it is read by are likely ever changing, meaning that the interpretation of a piece of information and its output (potentially phenotypic alteration) could also be constantly changing (Figure 4(B)). As a result, it becomes unreasonable to assume that we can easily follow information itself.

Ultimately, what is important to appreciate from this is that the absence of nongenetic carriers or phenotype reflective of the parental state cannot be taken as evidence that nongenetic information was not inherited. It may be the case that simply the information is now present in the offspring in a different carrier or is being interpreted in an alternative way. Therefore, when assessing the role of nongenetic information in evolution, the requirement for inheritance will potentially remain difficult to fulfill by the provision of direct evidence.

With this in mind, it can be beneficial to re-consider the dynamics of nongenetic information using an information centered approach. Information is a challenging concept in the context of biological systems and so it can be useful to approach the concept in alternative ways. Danchin et al. form analogies between information carriers and ‘avatars’, for instance, the DNA sequence can be considered an avatar of genetic information (Danchin 2013). They propose that the properties intrinsic to these avatars can influence the inheritance and interpretation of the information they carry, consistent with the statements we have made above. Interestingly, the authors reason that with this in mind, it is plausible to suggest that a selective pressure will exist for information to be encoded by a carrier with the optimal properties for a particular situation. It follows that there may be correlations between the mode of information storage and the nature of the environmental change or variation encoded by nongenetic information. For example, information relating to an environmental stimulus which is extremely stable would be selected to be encoded in DNA due to the properties of this carrier. In contrast, information about fluctuating environmental conditions may be favored to be encoded at the nongenetic level in a carrier which provides superior flexibility over short time scales. Ultimately, this approach supports the ideas proposed above, predicting that the role of nongenetic information in evolution will be heavily influenced and often facilitated by the properties of the carriers involved.

**Evolution at a different level**

Until now, the focus of this review has centered around the role of nongenetic information in the evolution of multicellular or unicellular organisms. However, it is also possible to draw parallels between these evolutionary processes and processes which occur within multicellular organisms. Specifically, populations of cells
Figure 4. The dynamics of nongenetic information: an information-based approach. (A) Nongenetic information can be stored in carriers and also transferred to alternative carriers. (B) The phenotypic output may vary depending on content. For instance, (i) if the information is read by different interpreters this may lead to a different output, or (ii) the integration of multiple pieces of nongenetic information may result in a different phenotype to that produced by only one piece of information. (C) The dynamics of nongenetic information must be considered when exploring nongenetic inheritance. (i) During sexual reproduction many nongenetic information carriers are destroyed during reprogramming. However, this does not necessarily mean that nongenetic information cannot be inherited. For instance, nongenetic information may be transferred to an alternative carrier in the gamete which is able to persist throughout embryo development and thus be inherited by the offspring. Even at this stage, the information may be transferred to another alternative carrier. (ii) During cell division nongenetic information carriers may be asymmetrically inherited, enabling nongenetic information to be rapidly lost in one of the two lineages. However, tracking the inheritance of a certain nongenetic information carrier may not reflect the inheritance path of nongenetic information due to potential transfer events between cell divisions. Therefore, it is difficult to draw conclusions about the extent of information inheritance purely through measurement of the information carriers themselves.
within multicellular organisms could be considered analogous to the populations of organisms already discussed. It has been recognized for many decades that populations of cells are able to change phenotypically, and thus can ‘evolve’ (Chisholm et al. 2016). The application of principles from evolutionary theory to these cells can be insightful for understanding these populations and, similarly, knowledge gained from studying these cell populations from an evolutionary perspective can provide novel outlooks on the evolution of multicellular organisms themselves. In particular, there are a number of advantages for using cell populations to explore evolutionary processes. Notably, cell populations offer a significantly reduced generation time compared to multicellular organisms, hence evolutionary processes are also able to be identified and measured on a more plausible timescale, which is often a barrier to evolutionary studies. Moreover, the reduced complexity of the system and recent technological advances in cell and molecular biology enable a vast amount of data to be collected and analyzed, far surpassing the information gained from traditional studies on multicellular organisms. In particular, there are a number of advantages offered when specifically considering the role of nongenetic information in evolution. Notably, the cells within a multicellular organism are largely similar in terms of genetic information, bar a few exceptions, enabling the contribution of nongenetic information to be isolated and identified in the absence of extensive genetic variation. Furthermore, the use of cell lines offers the possibility for clonal expansion of single cells and therefore the ability to work with genetically homogeneous populations containing many millions of individuals. Importantly, this provides an opportunity to assess the function of nongenetic information wholly independent of any genetic variation. Overall, the analysis of nongenetic information at the level of single cells is likely to enable us to more fully appreciate its role in evolution and integrate this into established evolutionary theories.

A major limitation when applying understanding gained from studies on single cells to populations of multicellular organisms, particularly in the context of evolution, is the dissimilarity in reproduction and inheritance. Production of offspring in a cell population simply requires the division of a mother cell into two daughter cells, akin to many unicellular organisms. Therefore, the challenges associated with inheritance in many multicellular organisms described above, especially in terms of nongenetic information, are not comparable. On the one hand, this reduced complexity potentially aids understanding of the evolution of cell populations, but nevertheless limits the transferability of evolutionary theories between this system and populations of multicellular organisms. Despite this, there are a number of similarities between the two systems which do enable comparisons to be made when considering evolution. Firstly, significant phenotypic variation can be found in cell populations, often arising from nongenetic sources (Kaern et al. 2005; Chang et al. 2008). In a similar way to previous discussions concerning multicellular organisms, this phenotypic variation can contribute to fitness and is therefore proposed to facilitate the evolution of these populations (Brock et al. 2009; Charlebois 2015).

Along these lines, there are a number of specific examples of evolutionary processes in cell populations, in which nongenetic information is thought to play a significant role. Firstly, multicellular organisms which undergo sexual reproduction, in addition to many which undergo asexual reproduction, begin from a single cell and over the course of development generate vast phenotypic diversity to form the numerous component cell types. This variation is produced whilst maintaining genetic homogeneity to a certain extent, inferring that this phenotypic change and resultant variation is underpinned by nongenetic information. Therefore, this process of differentiation, integral to developmental programs in order to generate phenotypically distinct cell types, could be considered as a model for evolutionary change driven by nongenetic mechanisms. Interestingly, it is thought that a number of the concepts discussed previously are key to the ‘evolution’ of cells during development in addition to the generation of nongenetic heterogeneity observed as phenotypic variation between cell types, in particular, phenotypic plasticity of individual cells. Importantly, the process of development results in numerous populations of cells occupying precise niches and displaying phenotypic specialization to function optimally. Leading up to this, the trajectory taken by each cell and its population is primarily determined by external signals originating from the environment it occupies and the plastic responses generated. Along these lines, the multicellular organism as a whole is itself the ‘environment’ within which individual cells and cell populations are found, analogous to a multicellular organism occupying a particular environment on Earth. With this view, it could be proposed that the difference between these two situations is that the environment experienced by cell populations produces stimuli and signals which are much more regulated in order to drive defined evolutionary trajectories to generate specific phenotypic states. The existence of numerous
different niches within a multicellular organism enables the production of many specialized cell populations. Overall, it appears that an organism as a whole determines the evolutionary trajectories of its component cell populations during the course of development to produce a functioning multicellular organism. In this way, it could be suggested that the initial appearance of multicellular organisms in history required cells to exploit key evolutionary processes, specifically those employing nongenetic mechanisms, to mold cooperative populations of cells with specialised functions necessary to make this critical evolutionary transition.

In this light, a key aspect to consider is that this phenotypic heterogeneity is produced from one single cell, and is generated within the time taken for development, which is relatively short when considering evolutionary time scales. Notably, this emphasizes the significant role that nongenetic information can play, even over short periods of time. Additionally, this process reflects situations in which populations of multicellular organisms are reduced to a minimal number of individuals and genetic variation is extremely limited. As described previously, under these circumstances it is crucial for increased phenotypic heterogeneity to be generated to improve the chances that this population can survive and propagate, which is suggested to be underpinned by nongenetic information. Similarly, during homeostasis of tissues in an adult organism, individual stem cells are required to generate phenotypically heterogeneous lineages via changes in nongenetic information alone. Therefore, evolutionary processes such as the generation of diversity after genetic bottlenecks and the founder effect can be found within multicellular organisms at the level of individual cells, which strengthens the argument for the role of nongenetic information in the evolution of multicellular organisms.

The evolution of cells within a multicellular organism is particularly evident when considering the progression of cancer. Historically it was accepted that cancer cell adaptation and evolution was driven by genetic changes, however the contribution of nongenetic mechanisms is becoming increasingly appreciated (Marine et al. 2020). In particular, analysis of some tumors has shown that adaptation driven by selection of genetic mutations ceases early on and further adaptation requires nongenetic change, via processes such as phenotypic plasticity (Williams et al. 2016). Moreover, there is evidence to suggest that cancers may exploit certain evolutionary strategies, such as bet-hedging, enabling them to persist in the presence of environmental change or therapeutic challenges (Chisholm et al. 2016; Bell and Gilan 2020). In particular, it has been shown in multiple cancer cell lines that there are small subpopulations of slow growing drug-tolerant persister cells (Sharma et al. 2010). This is highly reminiscent of the bet-hedging strategy identified in bacterial populations and is thought to represent a transiently acquired phenotype, maintained by activation of specific signaling pathways and an altered chromatin state. These subpopulations present a key area of interest when considering therapy resistance and relapse.

As discussed above, phenotypic plasticity is thought to be a key adaptive process in populations of multicellular organisms. Interestingly, data suggests that this process is also crucial to cancer cell populations during metastatic dissemination. Metastasis and colonization of a new site is analogous to the founder effect, and it is likely that plasticity of founding cells in response to the novel environment will facilitate rapid adaptation to these conditions and production of the phenotypic heterogeneity typical of cancer cell populations (Teeuwssen and Fodde 2019).

Overall, it is becoming increasingly clear that there is a significant role for nongenetic information in the evolutionary progression of tumors. Perhaps furthering our understanding of this process, particularly in terms of nongenetic mechanisms, will provide valuable insight to inform future therapeutic strategies.

Concluding remarks

In light of modern technological and conceptual advances, nongenetic information is now appreciated to make an important contribution to cellular function and identity. Nevertheless, the suggestion that this information may play a significant role in evolutionary processes has been met with strong opposition throughout history, largely a reflection of a lack of understanding and the belief that it opposed the widely accepted theories held in high regard at the time. In this review we have provided a framework to begin approaching these ideas from an alternative perspective, moving beyond the simplified view provided by considering solely common epigenetic modifications. Instead, focusing on nongenetic information itself could overcome conceptual barriers we believe are now limiting the potential reach of the vast amount of data generated by novel technologies.

Importantly, the ideas discussed in this review should be taken into consideration when reevaluating evolutionary theory in order to form a more complete synthesis moving forward. These concepts have far reaching implications in many fields of research, in addition to evolutionary biology. Strikingly, the basic...
principles of these ideas have been discussed for many years; now is the time to begin to fully appreciate them.

Acknowledgments

The authors thank Valeria Pavet for critical reading of the manuscript. YS is a CRUK-MI postdoctoral fellow, KLM is a recipient of the CRUK-MI PhD studentship and MMP is a CRUK-MI Fellow.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was funded by CRUK-MI core grant number A27412.

ORCID

Katherine L. Moran https://orcid.org/0000-0003-2353-7858
Maximiliano M. Portal https://orcid.org/0000-0002-2884-6900

References

Allendorf FW, Lundquist LL. 2003. Introduction: population biology, evolution, and control of invasive species. Conserv Biol. 17(1):24–30.

Ammar R, Torti D, Tsui K, Gebbia M, Durbic T, Bader GD, Giaever G, Nislow C. 2012. Chromatin is an ancient innovation conserved between Archaea and Eukarya. eLife. 1: e00078.

Arcos-Burgos M, Muenke M. 2002. Genetics of population isolates. Clin Genet. 61(4):233–247.

Aristotle 1910. Historia animalium. Translated by D’Arcy Wentworth Thompson. Oxford: The Clarendon Press.

Arzate-Mejia RG, Valle-Garcia D, Recillas-Targa F. 2011. Signaling epigenetics: novel insights on cell signaling and epigenetic regulation. IUBMB Life. 63(10):881–895.

Ashe A, Sapetschnig A, Weick EM, Mitchell J, Bagijn MP, Cording AC, Doebley AL, Goldstein LD, Lehrbach NJ, Le Pen J, et al. 2012. piRNAs can trigger a multigenerational epigenetic memory in the germline of C. elegans. Cell. 150(1):88–99.

Attwater J, Wochner A, Holliger P. 2013. In–ice evolution of RNA polymerase ribozyme activity. Nat Chem. 5(12):1011–1018.

Badyaev AV. 2014. Epigenetic resolution of the ‘curse of complexity’ in adaptive evolution of complex traits. J Physiol. 592(11):2251–2260.

Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. 2004. Bacterial persistence as a phenotypic switch. Science. 305(5690):1622–1625.

Baldwin MJ. 1896. A new factor in evolution. Am Naturalist. 30(354):441–451.

Beisson J, Sonneborn TM. 1965. Cytoplasmic inheritance of the organization of the cell cortex in paramecium aurelia. Proc Natl Acad Sci USA. 53:275–282.

Beisson J. 2008. Preformed cell structure and cell heredity. Prion. 2(1):1–8.

Bell CC, Gilan O. 2020. Principles and mechanisms of non-genetic resistance in cancer. Br J Cancer. 122(4):465–472.

Bell MA, Travis MP, Blouw DM. 2006. Inferring natural selection in a fossil threespine stickleback. Paleobiology. 32(4):562–577.

Bewick AJ, Hofmeister BT, Powers RA, Mondo SJ, Grigoriev IV, James TY, Stajich JE, Schmitz RJ. 2019. Diversity of cytosine methylation across the fungal tree of life. Nat Ecol Evol. 3(3):479–489.

Bonduriansky R, Day T. 2009. Nongenetic inheritance and its evolutionary implications. Annu Rev Ecol Evol Syst. 40(1):103–125.

Boskovic A, Rando OJ. 2018. Transgenerational epigenetic inheritance. Annu Rev Genet. 52:21–41.

Brock A, Chang H, Huang S. 2009. Non-genetic heterogeneity – a mutation-independent driving force for the somatic evolution of tumours. Nat Rev Genet. 10(5):336–342.

Burton T, Metcalfe NB. 2014. Can environmental conditions experienced in early life influence future generations? Proc Biol Sci. 281(1785):20140311.

Chahwan R, Wontakal SN, Roa S. 2010. Crosstalk between genetic and epigenetic information through cytosine deamination. Trends Genet. 26(10):443–448.

Chang HH, Hemberg M, Barahona M, Ingber DE, Huang S. 2008. Transcriptome-wide noise controls lineage choice in mammalian progenitor cells. Nature. 453(7194):544–547.

Charlebois DA. 2015. Effect and evolution of gene expression noise on the fitness landscape. Phys Rev E Stat Nonlin Soft Matter Phys. 92(2):022713.

Chen Q, Yan M, Cao Z, Li X, Zhang Y, Shi J, Feng GH, Peng H, Zhang X, Zhang Y, et al. 2016. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. Science. 351(6271):397–400.

Chen Q, Yan W, Duan E. 2016. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. Nat Rev Genet. 17(12):733–743.

Chisholm RH, Lorenzi T, Clairambault J. 2016. Cell population heterogeneity and evolution towards drug resistance in cancer: Biological and mathematical assessment, theoretical treatment optimisation. Biochim Biophys Acta. 1860(11 Pt B):2627–2645.

Clune J. 2019. AI-GAs: AI-generating algorithms, an alternate paradigm for producing general artificial intelligence. ArXiv. abs/1905.10985.

Condorcet J-A-N. 1794. Esquisse d’un tableau historique des progrès de l’esprit humain: ouvrage posthume de Condorcet. Paris: Agasse.

Danchin E. 2013. Avatars of information: towards an inclusive evolutionary theory of evolution. Nat Rev Genet. 14(7):475–486.

Danchin E. 2013. Avatars of information: towards an inclusive evolutionary theory of evolution. Nat Rev Genet. 14(7):475–486.

Danchin E. 2013. Avatars of information: towards an inclusive evolutionary theory of evolution. Nat Rev Genet. 14(7):475–486.
Darwin C, Kebler L. 1859. On the origin of species by means of natural selection, or, The preservation of favoured races in the struggle for life. London: J. Murray.

Darwin C. 1868. The variation of animals and plants under domestication. London: John Murray.

Day T, Bonduriansky R. 2011. A unified approach to the evolutionary consequences of genetic and nongenetic inheritance. Am Nat. 178(2):E18–E36.

De Meyer J, Christiaens J, Adriaens D. 2016. Diet-induced phenotypic plasticity in European eel (Anguilla anguilla). J Exp Biol. 219(Pt 3):354–363.

Deo RC. 2015. Machine Learning in Medicine. Circulation. 132(20):1920–1930.

Dias BG, Ressler KJ. 2014. Parental olfactory experience influences behavior and neural structure in subsequent generations. Nat Neurosci. 17(1):89–96.

Dunwell TL, Pfeifer GP. 2014. Drosophila genomic methylation: new evidence and new questions. Epigenomics. 6(5):459–461.

Feng S, Cokus SJ, Zhang X, Chen PY, Bostick M, Goll MG, Hetzel J, Jain J, Strauss SH, Halpern ME, et al. 2010. Conservation and divergence of methylation patterning in plants and animals. Proc Natl Acad Sci USA. 107(19):8689–8694.

Fields C, Levin M. 2018. Multiscale memory and bioelectric error correction in the cytoplasm-cytoskeleton-membrane system. Wiley Interdiscip Rev Syst Biol Med. 10(2):e1410.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 391(6669):806–811.

Focher F. 2014. Maupertuis: the ‘old synthesis’. J Genet. 93(2):607–608.

Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J, Farinelli L, Miska E, Mansuy IM. 2014. Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nat Neurosci. 17(5):667–669.

Geoghegan JL, Spencer HG. 2012. Population-epigenetic models of selection. Theor Popul Biol. 81(3):232–242.

Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA. 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. Nature. 525(7569):372–375.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct Ecol. 21(3):394–407.

Gibbs HL, Grant PR. 1987. Ecological Consequences of an Exceptionally Strong El Nino Event on Darwin’s Finches. Ecology. 68(6):1735–1746.

Glass HB. 1955. Maupertuis, a Forgotten Genius. Sci Am. 193(4):100–111.

Goldblatt J, Minutillo C, Pemberton PJ, Hurst J. 1992. Ellis-van Creveld syndrome in a Western Australian aboriginal community. Postaxial polydactyly as a heterozygous manifestation? Med J Aust. 157(4):271–272.

Goll MG, Bestor TH. 2005. Eukaryotic cytosine methyltransferases. Annu Rev Biochem. 74:481–514.

Greer EL, Blanco MA, Gu L, Sendinc E, Liu J, Aristizabal-Corrales D, Hsu CH, Aravind L, He C, Shi Y. 2015. DNA Methylation on N6-Adenine in C. elegans. Cell. 161(4):868–878.

Hamet P, Tremblay J. 2017. Artificial intelligence in medicine. Metabolism. 69S:S36–S40.

Harrison LB, Yu Z, Stajich JE, Dietrich FS, Harrison PM. 2007. Evolution of budding yeast prion-determinant sequences across diverse fungi. J Mol Biol. 368(1):273–282.

Heard E, Martienssen RA. 2014. Transgenerational epigenetic inheritance: myths and mechanisms. Cell. 157(1):95–109.

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey LH. 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A. 105(44):17046–17049.

Herman JJ, Spencer HG, Donohue K, Sultan SE. 2014. How stable ‘should’ epigenetic modifications be? Insights from adaptive plasticity and bet hedging. Evolution. 68(3):632–643.

Herrera CM, Bazaga P. 2011. Untangling individual variation in natural populations: ecological, genetic and epigenetic correlates of long-term inequality in herbivory. Mol Ecol. 20(8):1675–1688.

Higgs PG, Lehman N. 2015. The RNA World: molecular cooperation at the origins of life. Nat Rev Genet. 16(1):7–17.

Hiyoshi A, Miyahara K, Kato C, Ohshima Y. 2011. Does a DNA-less cellular organism exist on Earth? Genes Cells. 16(12):1146–1158.

Holeski LM, Jander G, Agrawal AA. 2012. Transgenerational defense induction and epigenetic inheritance in plants. Trends Ecol Evol. 27(11):618–626.

Holland J, Spindler K, Horodyński F, Grabau E, Nichol S, VandePol S. 1982. Rapid evolution of RNA genomes. Science. 215(4540):1577–1585.

Houri-Zeevi L, Korem Kohanim Y, Antonova O, Rechavi O. 2020. Three rules explain transgenerational small RNA inheritance in C. elegans. Cell. 182(5):1186–1197 e1112.

Huxley J. 1942. Evolution: The Modern Synthesis. London: Allen & Unwin.

Immler S. 2018. The sperm factor: paternal impact beyond genes. Heredity (Edinb). 121(3):239–247.

Jablonka E. 2013. Epigenetic inheritance and plasticity: The responsive germline. Prog Biophys Mol Biol. 111(2-3):99–107.

Jablonka E. 2017. The evolutionary implications of epigenetic inheritance. Interface Focus. 7(5):20160135

Johannes F, Colot V, Jansen RC. 2008. Epigenome dynamics: a quantitative genetics perspective. Nat Rev Genet. 9(11):883–890.

Kaern M, Elston TC, Blake WJ, Collins JJ. 2005. Stochasticity in gene expression: from theories to phenotypes. Nat Rev Mol Cell Biol. 6:451–464.

Kimura M. 1979. The neutral theory of molecular evolution. Sci Am. 241(5):98–100, 102, 108 passim.

Kirk GSR, Schofield M. 1983. The presocratic philosophers: a critical history with a selection of texts. 2, revised ed. Cambridge (UK): Cambridge University Press.

Kussell E, Kishony R, Balaban NQ, Leibler S. 2005. Bacterial persistence: a model of survival in changing environments. Genetics. 169(4):1807–1814.

Lachmann M, Jablonka E. 1996. The inheritance of phenotypes: an adaptation to fluctuating environments. J Theor Biol. 181(1):1–9.
Laland K, Uller T, Feldman M, Sterelny K, Muller GB, Moczek A, Jablonka E, Odling-Smee J, Wray GA, Hoekstra HE, et al. 2014. Does evolutionary theory need a rethink? Nature. 514(7521):161–164.

Lamark J-B. 1815. Histoire naturelle des animaux sans vertèbres. Paris: Verdière.

Langerhans RB, DeWitt TJ. 2002. Plasticity constrained: overgeneralized induction cues cause maladaptive phenotypes. Evolutionary Ecology Research. 4:857–870.

Lazzcano A, Guerrero R, Margulis L, Oro J. 1988. The evolutionary transition from RNA to DNA in early cells. J Mol Evol. 27(4):283–290.

LeRoy C-G. 1802. Lettres philosophiques sur l'intelligence et la perfectibilité des animaux, avec quelques lettres sur l'homme. Paris: Bossange, Masson et Besson.

Levy SF. 2016. Cellular heterogeneity: benefits besides bet-hedging. Curr Biol. 26(9):R355–357.

Liebl AL, Schrey AW, Richards CL, Martin LB. 2013. Patterns of DNA methylation throughout a range expansion of an introduced songbird. Integr Comp Biol. 53(2):351–358.

Lincoln TA, Joyce GF. 2009. Self-sustained replication of an introduced songbird. Integr Comp Biol. 53(2):351–358.

Lyko F, Maleszka R. 2011. Insects as innovative models for stress activated retrotransposons on genome evolution in Archaea and Eukarya: a comparative analysis. Extremophiles. 2(3):141–148.

Pérez JE, Nirchio M, Alfonsi C, Muñoz C. 2006. The biology of invasions: the genetic adaptation paradox. Biol Invasions. 8(5):1115–1121.

Lazcano A, Guerrero R, Margulis L, Oro J. 1988. The evolutionary transition from RNA to DNA in early cells. J Mol Evol. 27(4):283–290.

LeRoy C-G. 1802. Lettres philosophiques sur l'intelligence et la perfectibilité des animaux, avec quelques lettres sur l'homme. Paris: Bossange, Masson et Besson.

Levy SF. 2016. Cellular heterogeneity: benefits besides bet-hedging. Curr Biol. 26(9):R355–357.

Liebl AL, Schrey AW, Richards CL, Martin LB. 2013. Patterns of DNA methylation throughout a range expansion of an introduced songbird. Integr Comp Biol. 53(2):351–358.

Lincoln TA, Joyce GF. 2009. Self-sustained replication of an introduced songbird. Integr Comp Biol. 53(2):351–358.

Lyko F, Maleszka R. 2011. Insects as innovative models for functional studies of DNA methylation. Trends Genet. 27(4):127–131.

Margueron R, Reinberg D. 2010. Chromatin structure and the inheritance of epigenetic information. Nat Rev Genet. 11(4):285–296.

Marine JC, Dawson SJ, Dawson MA. 2020. Non-genetic mechanisms of therapeutic resistance in cancer. Nat Rev Cancer. 20(12):743–756.

Maumus F, Allen AE, Mhiri C, Hu H, Jabbari K, Vardi A, Grandbastien MA, Bowler C. 2009. Potential impact of stress activated retrotransposons on genome evolution in a marine diatom. BMC Genomics. 10:624

Maupertuis P-L. 1751b. Système de la Nature. In: OEuvres, 1756. Vol. 2. Lyon: J-M. Bruyset.

McNew SM, Beck D, Sadler-Riggleman I, Knutie SA, Koop JAH, Clayton DH, Skinner MK. 2017. Epigenetic variation between urban and rural populations of Darwin’s finches. BMC Evol Biol. 17(1):183.

Merila J, Hendry AP. 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Evol Appl. 7(1):1–14.

Miroouze M, Vitte C. 2014. Transposable elements, a treasure trove to decipher epigenetic variation: insights from Arabidopsis and crop epigenomes. J Exp Bot. 65(10):2801–2812.

Miska EA, Ferguson-Smith AC. 2016. Transgenerational inheritance: models and mechanisms of non-DNA sequence-based inheritance. Science. 354(6308):59–63.

Mostowy R, Engelstädter J, Salathe M. 2012. Non-genetic inheritance and the patterns of antagonistic coevolution. BMC Evol Biol. 12:93.

O’Dea RE, Noble DWA, Johnson SL, Hesselson D, Nakagawa S. 2016. The role of non-genetic inheritance in evolutionary rescue: epigenetic buffering, heritable bet hedging and epigenetic traps. Environ Epigenet. 2(1):dvv014.

Paaby AB, Rockman MV. 2014. Cryptic genetic variation: evolution’s hidden substrate. Nat Rev Genet. 15(4):247–258.

Pal C, Miklos I. 1999. Epigenetic inheritance, genetic assimilation and speciation. J Theor Biol. 200(1):19–37.

Peng H, Shi J, Zhang Y, Zhang H, Liao S, Li W, Lei L, Han C, Ning L, Cao Y, et al. 2012. A novel class of RNA-derived small RNAs extremely enriched in mature mouse sperm. Cell Res. 22(11):1609–1612.

Pereira SL, Grayling RA, Lurz R, Reeve JN. 1997. Archaeal nucleosomes. Proc Natl Acad Sci USA. 94(23):12633–12637.

Pereira SL, Reeve JN. 1998. Histones and nucleosomes in Archaea and Eukarya: a comparative analysis. Extremophiles. 2(3):141–148.

Pérez JE, Nirchio M, Alfonsi C, Muñoz C. 2006. The biology of invasions: the genetic adaptation paradox. Biol Invasions. 8(5):1115–1121.

Pigliucci M, Murren CJ, Schlichting CD. 2006. Phenotypic plasticity and evolution by genetic assimilation. J Exp Biol. 209(Pt 12):2362–2367.

Ramchandani S, Bhattacharya SK, Cervoni N, Szyf M. 1999. DNA methylation is a reversible biological signal. Proc Natl Acad Sci USA. 96(11):6107–6112.

Ranganath HA. 2018. Darwin’s finches: a goldmine for evolutionary biologists. J Genet. 97(4):807–809.

Rastogi A, Lin X, Lombard B, Loew D, Trichine L, 1 Ecology and Evolutionary Biology Section, Institut de Biologie de l’Ecole Normale Supérieure (IBENS), CNRS UMR8197 INSERM U1024, 46 rue d’Ulm 75005 Paris, France 2015. Probing the evolutionary history of epigenetic mechanisms: what can we learn from marine diatoms. AIMS Genetics. 2(3):173–191.

Rechavi O, Lev I. 2017. Principles of transgenerational small RNA inheritance in Caenorhabditis elegans. Curr Biol. 27(14):R720–R730.

Rechavi O, Minevich G, Hobert O. 2011. Transgenerational inheritance of an acquired small RNA-based antiviral response in C. elegans. Cell. 147(6):1248–1256.

Relyea RA. 2002. Costs of phenotypic plasticity. Am Nat. 159(3):272–282.

Richards CL, Bossdorf O, Pigliucci M. 2010. What role does heritable epigenetic variation play in phenotypic evolution? BioScience. 60(3):232–237.

Richards CL, Schrey AW, Pigliucci M. 2012. Invasion of diverse habitats by few Japanese knotweed genotypes is correlated with epigenetic differentiation. Ecol Lett. 15(9):1016–1025.

Robertson MP, Joyce GF. 2014. Highly efficient self-replicating RNA enzymes. Chem Biol. 21(2):238–245.

Roemer I, Reik W, Dean W, Klose J. 1997. Epigenetic inheritance of an acquired small RNA-based antiviral response in C. elegans. Cell. 147(6):1248–1256.

Routledge RA. 2002. Costs of phenotypic plasticity. Am Nat. 159(3):272–282.

Richards CL, Bossdorf O, Pigliucci M. 2010. What role does heritable epigenetic variation play in phenotypic evolution? BioScience. 60(3):232–237.

Richards CL, Schrey AW, Pigliucci M. 2012. Invasion of diverse habitats by few Japanese knotweed genotypes is correlated with epigenetic differentiation. Ecol Lett. 15(9):1016–1025.

Robertson MP, Joyce GF. 2014. Highly efficient self-replicating RNA enzymes. Chem Biol. 21(2):238–245.

Roemer I, Reik W, Dean W, Klose J. 1997. Epigenetic inheritance in the mouse. Curr Biol. 7(4):277–280.

Roundtree IA, Evans ME, Pan T, He C. 2017. Dynamic RNA Modifications in Gene Expression Regulation. Cell. 169(7):1187–1200.

Sarker G, Sun W, Rosenkranz D, Pelczar P, Opitz L, Efthymiou V, Wolfrum C, Peleg-Raibstein D. 2019. Maternal overnutrition programs hedonic and metabolic phenotypes across generations through sperm tRNAs. Proc Natl Acad Sci USA. 116(21):10547–10556.

Sarkies P. 2020. Molecular mechanisms of epigenetic inheritance: possible evolutionary implications. Semin Cell Dev Biol. 97:106–115.
Sharma U, Conine CC, Shea JM, Boskovic A, Derr AG, Bing Shorter J, Lindquist S. 2005. Prions as adaptive conduits of Sentis A, Bertram R, Dardenne N, Ramon-Portugal F, Schuster A, Skinner MK, Yan W. 2016. Ancestral vinclozolin Schrey AW, Coon CA, Grispo MT, Awad M, Imboma T, McCoy Inboma T, McCoy et al. 2018. Epigenetic variation may compensate for decreased genetic variation with introductions: a case study using house sparrows (Passer domesticus) on two continents. Genet Res Int. 2012:1–7.

Schuster A, Skinner MK, Yan W. 2016. Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs. Environ Epigenet. 2(1):dww001.

Sentis A, Bertram R, Dardenne N, Ramon-Portugal F, Espinasse G, Louit I, Negri L, Haeler A, Ashkar T, Pannetier T, et al. 2018. Evolution without standing genetic variation: change in transgenerational plastic response under persistent predation pressure. Heredity (Edinb)). 121(3):266–281.

Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, McDermott U, Azizian N, Zou L, Fischbach MA, et al. 2010. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell. 141(1):69–80.

Sharma U, Conine CC, Shea JM, Boskovic A, Derr AG, Bing XY, Belleannce C, Kucukural A, Serra RW, Sun F, et al. 2016. Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. Science. 351(6271):391–396.

Shyakhutina Y, Moran KL, Portal MM. 2019. Asymmetric inheritance of cell fate determinants: focus on RNA. Noncoding RNA. 5(2):38.

Shorter J, Lindquist S. 2005. Prions as adaptive conduits of memory and inheritance. Nat Rev Genet. 6(6):435–450.

Sigman MJ, SLOTKIN RK. 2016. The first rule of plant transposable element silencing: location, location, location. Plant Cell. 28(2):304–313.

Skvortsova K, Lovino N, Bogdanovic O. 2018. Functions and mechanisms of epigenetic inheritance in animals. Nat Rev Mol Cell Biol. 19(12):774–790.

Slotkin RK, Martienssen R. 2007. Transposable elements and the epigenetic regulation of the genome. Nat Rev Genet. 8(4):272–285.

Sommer RJ, Dardiry M, Lenucci M, Namdeo S, Renahan T, Sieriebrennikov B, Werner MS. 2017. The genetics of phenotypic plasticity in nematode feeding structures. Open Biol. 7(3):160332.

Teeuwen M, Fodde R. 2019. Cell heterogeneity and phenotypic plasticity in metastasis formation: the case of colon cancer. Cancers. 11(9):1368.

Trevisanato SL. 2016. Reconstructing Anaximander’s biological model unveils a theory of evolution akin to Darwin’s, though centuries before the birth of science. Acta Med Hist Adriat. 14(1):63–71.

True HL, Berlin I, Lindquist SL. 2004. Epigenetic regulation of translation reveals hidden genetic variation to produce complex traits. Nature. 431(7005):184–187.

True HL, Lindquist SL. 2000. A yeast prion provides a mechanism for genetic variation and phenotypic diversity. Nature. 407(6803):477–483.

Van Kleunen M, Fischer M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. New Phytologist. 166(1):49–60.

Wenendaal MV, Painter RC, de Rooij SR, Bossuyt PM, van der Post JA, Gluckman PD, Hanson MA, Roseboom TJ. 2013. Transgenerational effects of prenatal exposure to the 1944–45 Dutch famine. BJOG. 120(5):548–553.

Waddington CH. 1942a. Canalization of development and the inheritance of acquired characters. Nature. 150(3811):563–565.

Waddington CH. 1942b. The epigenotype. Endeavor. 1:18–20.

Walker JA. 1997. Ecological morphology of lacustrine threespine stickleback Gasterosteus aculeatus L. (Gasterosteidae) body shape. Biol J Linnean Soc. 61(1):3–50.

Watson RA, Szathmary E. 2016. How can evolution learn? Trends Ecol Evol. 31(2):147–157.

West-Eberhard MJ. 2005. Developmental plasticity and the origin of species differences. Proc Natl Acad Sci USA. 102(Suppl 1):5643–5649.

Williams MJ, Werner B, Barnes CP, Graham TA, Sotorava A. 2016. Identification of neutral tumor evolution across cancer types. Nat Genet. 48(3):238–244.

Wimberger PH. 1992. Plasticity of fish body shape. The effects of diet, development, family and age in two species of Geophagus (Pisces: Cichlidae). Biol J Linnean Soc. 45(3):197–218.

Wintzer AP, Motta PJ. 2005. Diet-induced phenotypic plasticity in the skull morphology of hatchery-reared Florida largemouth bass, Micropterus salmoides floridanus. Ecol Freshwater Fish. 14(4):311–318.

Yehuda R, Daskalakis NP, Bierer LM, Bader HN, Klengel T, Wintzer AP, Motta PJ. 2005. Diet-induced phenotypic plasticity in the skull morphology of hatchery-reared Florida largemouth bass, Micropterus salmoides floridanus. Ecol Freshwater Fish. 14(4):311–318.

Yehuda R, Daskalakis NP, Bierer LM, Bader HN, Klengel T, Wintzer AP, Motta PJ. 2005. Diet-induced phenotypic plasticity in the skull morphology of hatchery-reared Florida largemouth bass, Micropterus salmoides floridanus. Ecol Freshwater Fish. 14(4):311–318.

Yoder JA, Walsh CP, Bestor TH. 1997. Cytosine methylation and the ecology of intragenomic parasites. Trends Genet. 13(8):335–340.

Zemach A, McDaniel IE, Silva P, Zilberman D. 2010. Genomewide evolutionary analysis of eukaryotic DNA methylation. Science. 328(5980):916–919.

Zemach A, Zilberman D. 2010. Evolution of eukaryotic DNA methylation and the pursuit of safer sex. Curr Biol. 20(17):R780–785.

Zhang Y, Chen Q. 2019. The expanding repertoire of hereditary information carriers. Development. 146(6):dev170902.

Zhang Y, Shi J, Rassoulzadegan M, Tuorto F, Chen Q. 2019. Sperm RNA code programmes the metabolic health of offspring. Nat Rev Endocrinol. 15(8):489–498.

Zhang Y, Zhang X, Shi J, Tuorto F, Li X, Liu Y, Liebers R, Zhang L, Qu Y, Qian J, et al. 2018. Dnmt2 mediates intergenerational transmission of paternally acquired metabolic disorders through sperm small non-coding RNAs. Nat Cell Biol. 20(5):535–540.