How epigenetic mutations can affect genetic evolution: Model and mechanism

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We hypothesize that heritable epigenetic changes can affect rates of fitness increase as well as patterns of genotypic and phenotypic change during adaptation. In particular, we suggest that when natural selection acts on pure epigenetic variation in addition to genetic variation, populations adapt faster, and adaptive phenotypes can arise before any genetic changes. This may make it difficult to reconcile the timing of adaptive events detected using conventional population genetics tools based on DNA sequence data with environmental drivers of adaptation, such as changes in climate. Epigenetic modifications are frequently associated with somatic cell differentiation, but recently epigenetic changes have been found that can be transmitted over many generations. Here, we show how the interplay of these heritable epigenetic changes with genetic changes can affect adaptive evolution, and how epigenetic changes affect the signature of selection in the genetic record.

Keywords:
adaptive walks; epigenetics; methylation; non-genetic inheritance

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Introduction

Epigenetic changes, such as DNA methylation or histone modifications, play a central role in cell differentiation. They enable cells that are genetically nearly identical to differentiate into a myriad of different cell types. While these modifications are inherited by daughter cells during cell division, such changes are generally not transmitted from one generation to the next through sexual reproduction. Instead, they are usually ''reset'' at each generation. However, there is evidence that some epigenetic changes can escape the usual ''reset'', and persist through sexual reproduction. This has been documented for a wide range of epigenetic mechanisms and organisms [1–7]. These mechanisms include cytosine methylation, histone acetylation, micro-RNAs, and any other molecules that have the potential to modulate the relationship between genome and phenotype, and have been grouped together by function into the ''interpretive machinery'' of the genome [8]. If changes to the interpretive machinery of the genome, including epigenetic changes such as methylation states, can be transmitted across generations, they can in principle also affect adaptation [1, 9, 10]. Epigenetic changes, alongside other kinds of non-genetic inheritance such as parental effects, ecological inheritance, and cultural inheritance, then have the potential to influence the fitness gain of a population, the nature of adaptive phenotypes, and the pattern of genetic change that eventually underlies adaptive evolution [8, 11]. Here, we model evolution on a combined genetic/epigenetic fitness landscape to explore how the inheritance of pure epigenetic change (*sensu* Richards [12]) can affect adaptation through the interplay of genetic and epigenetic variation during an adaptive walk [8, 13]. We find that when pure epigenetic variation and genetic variation are both acted on by natural selection, adapted phenotypes can show up long before genetic changes do, and populations can adapt faster than in cases where natural selection acts only on genetic variation. These hypotheses can be tested in evolution experiments that we discuss.

Several molecular mechanisms can produce heritable epigenetic mutations

For natural selection to act on pure epigenetic variation in a way that results in adaptation, this variation needs to be heritable. Several mechanisms can produce heritable epigenetic variation. One extensively studied example of epigenetic inheritance is paramutation, where the combination of two alleles permits a heritable change in gene expression. Here, one of the alleles associated with a transcriptionally silent gene causes the silencing of a gene associated with the other allele. Initially found in maize more than 50 years ago [14], paramutations have been found across a wide range of plant species [2] and in mice [15]. The functional basis of paramutations is not clear, but chromatin modifications and small non-coding RNA may play a role [2].

Small RNAs are another mechanism for transmitting non-genetic information between generations. Recent studies in *C. elegans* have demonstrated that RNA interference (RNAi) can maintain epigenetic changes over multiple generations. Worms were injected with a double-stranded RNA targeting a gene via RNAi. Following this, gene silencing persisted in populations indefinitely and in the absence of the initial stimulus [3]. In a second study, a piwi-interacting RNA induced gene silencing over at least 24 generations [16]. In both cases, a particular Argonaute protein is required to maintain silencing [4], and a number of chromatin factors were identified in the second study. For a recent review of the roles that small RNAs can play in transgenerational epigenetic inheritance, see Castel and Martienssen [7].

Another mechanism that has been implied in transgenerational epigenetic inheritance is methylation, even though it is not known how a particular subset of methylation marks are preserved during meiosis instead of being ''reset'' along with the others. In rats, a single dose of a fungicide (Vinclozolin, an androgen antagonist) given to pregnant females has been found to decrease male fertility for at least four generations [5, 17], and the epigenetic mechanism responsible for transmitting the effect is thought to be methylation patterns. A second study showed that in mice, susceptibility to testicular tumors can been found in offspring of a carrier of a null allele *deadend1* [6], and tumor susceptibility persisted for at least three generations even when *deadend1* had been restored by backcrossing. Since *deadend1* is thought to encode part of a cytidine deaminase, the authors suggested that the epigenetic effects from the lack of the deaminase persisted and caused tumors over several generations [6]. For reviews on transgenerational epigenetic inheritance, see [1, 2, 18].

In all of these cases, it is the epigenetic state itself that is passed from one generation to the next, not the genetic blueprint of how to set up a particular methylation pattern or chromatin configuration. In cases where this epigenetic state is independent of underlying genetic variation, it corresponds to ''pure epigenetic variation'' *sensu* Richards [12]. Of course not all epigenetic changes are heritable, the somatic epigenetic changes involved in cell differentiation being a case in point. Neither are all changes to DNA methylation, chromatin state or small interfering RNA in the germline heritable. While the proportion of heritable changes in epigenetic states is unknown, as is the proportion of heritable epigenetic variation that contributes to pure epigenetic variation, the examples above show that at least some epigenetic changes can be passed from one generation to the next even in the absence of underlying genetic changes. For the case studies involving RNAi, there is no limit on the function of target genes that can be silenced, and there is a dedicated molecular machinery for epigenetic inheritance. Here we ask: what can natural selection do with this mechanism?

Epigenetic mutations and evolution: Theoretical approaches

Recent studies have explored different ways to incorporate non-genetic inheritance, including epigenetic marks, parental effects, ecological inheritance, and cultural inheritance, into evolutionary theory [8, 11, 19–21]. In general, these studies categorize non-genetic inheritance by mechanism, and examples of mechanisms include epigenetic modification and other aspects of the ''interpretive machinery'' of a genome, ecological modifications, and between-generation learning. By doing this, particular molecular mechanisms can be explicitly incorporated into adaptive theory. For example, Geoghegan and Spencer [21] extend previous models on phenotypic plasticity by Kirkpatrick and Lande [19] to integrate epigenetically encoded plastic responses into population-epigenetic models of selection, and find that epialleles can maintain phenotypic variation in a changing environment, even in the absence of genetic variation. A second approach, taken by Bonduriansky and Day [8, 20], uses the Price equation to partition the contributions of non-genetic and genetic inheritance during evolution. This approach highlighted the potential for complex interactions between genetic alleles and epialleles such as epialleles impeding the fixation of beneficial genetic alleles.

Here we use a model that differs in approach and applicability from previous work discussed above in several ways. Models by Pal and Miklos and Pal consider non-genetic inheritance as part of an environmentally induced plastic response [22, 23], whereas we consider epigenetic variation that is independent of genetic variation, also called ''pure epigenetic variation'' [12]. Second, models by Bonduriansky and Day [8, 20] and Geoghegan and Spencer [21] investigate the contribution of non-genetic inheritance in the absence of genetic variation, or in the case of a single variable epigenetic locus and a single variable genetic locus [8, 20, 21]. In contrast, our model investigates how pure epigenetic inheritance affects adaptive walks where population fitness increases by the sequential substitution of beneficial variants in a population, and where mutations can arise de novo at multiple genetic and epigenetic loci. This allows us to investigate how pure epigenetic variation can speed up or slow down evolutionary trajectories on a variety of fitness landscapes.

Natural selection on epigenetic mutations: The effect of mutation rate

Individuals in a population differ in fitness, that is they differ in their expected number of offspring. Because fitter

individuals have more offspring than less fit ones, the average fitness of a population tends to increase over time: adaptation by natural selection. Differences in fitness between individuals are produced by mutations of some kind, and those mutations must be heritable for natural selection to act. While we are accustomed to think about mutations as changes in DNA sequence, any difference between individuals that is heritable and affects fitness can be acted on by natural selection [9, 24]. A number of molecular events fit this description; point mutations in DNA, transposition events, changes in methylation or acetylation patterns.

One key difference between these mechanisms is the degree to which a change is heritable: Given the state of a molecular system in an individual – the nucleotide at a particular locus or the methylation state at a particular chromosomal location – will the next generation inherit that state or not? This is quantified by the probability of a change from one generation to the next (mutation rate): For DNA the probability of a change per nucleotide per individual and per generation is of the order of 10^{-6} to 10^{-9} [25–27]. For the methylation state at a particular chromosomal position the methylation/demethylation probability per cytosine is of the order of 10^{-4} per CG pair [26], and is thus orders of magnitude larger than the DNA point mutation rate measured in the same experiment. For transcription silencing by RNAi in *Caenorhabditis elegans*, stable silencing has been observed over at least 24 generations [16]. Although there are not enough published data to estimate epigenetic mutation rates very well, it is safe to say that in general they are higher than genetic mutation rates.

Given the higher mutation rates associated with epigenetic mechanisms, one could argue that any adaptation achieved via an epigenetic mechanism might be lost again just as quickly as it arose. However, whether or not this is the case depends on the strength of selection. Consider alleles (genetic or epigenetic) with some fitness differences between the fittest allele and the other alleles and a mutation rate μ. Looking at the individuals with the fitter variant of the allele, a fraction μ of them will mutate to a less fit allele in each generation. However, the expected number of offspring is also higher for individuals bearing the fitter allele. Provided the fitness difference is large enough, the higher number of offspring can compensate the losses through mutations, so that the frequency of the fitter allele in the population remains high. This is the mechanism at the heart of the so-called error catastrophe in population genetics [28], where a population can only be kept at a fitness peak of height s provided the mutation rate remains less than s. This limits the evolutionary scenarios where we can expect pure epigenetic variation to play a role in the dynamics of adaptive walks: The fitness gain achieved must exceed the mutation rate (per generation) of the mechanism used to encode the new phenotype (see Fig. 1). However, populations whose adaptive phenotype is encoded by a system with a high mutation rate will have a higher mutation load, and thus slightly lower mean fitness, than a population that can encode the same phenotype with a higher-fidelity system. In our model, we take fixation to mean the case where most of the individuals in the population have the epigenetic (or genetic) locus in a particular state, and in this case, the population will have some polymorphism due to mutation load.

Here we focus only on differences in mutation rate between genetic and epigenetic mutations, even though there may also be differences in the distribution of fitness effects of genetic and epigenetic mutations. In particular, epigenetic mutations may be more likely to be beneficial than genetic mutations since they share a molecular mechanism with adaptive phenotypic plasticity. In addition, epigenetic mutations are likely to have a narrower range of effects (changing gene expression levels) than genetic mutations that can change gene expression levels, but also change gene products and rearrange genetic material, and there is some evidence that mutations in coding and non-coding DNA have different distributions of fitness effects [29]. However, few studies distinguish between epigenetic mutations that are genetically encoded and those that are not, and it is possible that any tendency that epigenetic mutations have to be beneficial stems from them being genetically encoded as part of an adaptive plastic response. Since there is little data on the fitness distribution of epigenetic mutations that make up pure epigenetic variation, we use the conservative assumption that epigenetic mutations

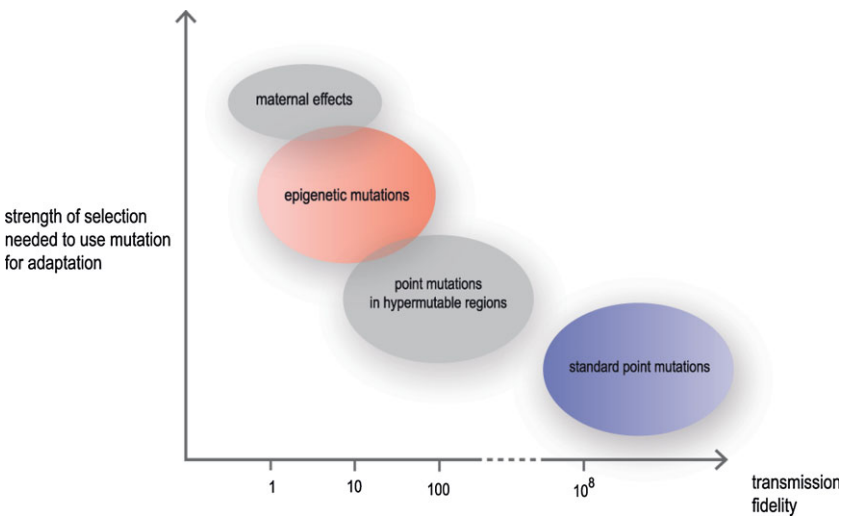

Figure 1. Different mechanisms that can generate heritable variance in fitness. Mechanisms are grouped by transmission fidelity (quantified by the expected number of generations between successive mutations), and the strength of selection required to act on traits encoded by each group. Under very strong selection, such as acute fitness loss when a population is exposed to a toxin, nearly any mechanism that produces heritable variance in fitness can produce mutants that natural selection acts upon. As a population adapts, the strength of selection will decrease as will the number of mechanisms with high enough transmission fidelities.

involved in pure epigenetic variation are, on average, no more likely to be beneficial than genetic mutations. In addition, our model focuses on phenotypes that can be reached using either genetic or pure epigenetic mutations, and so does not address the effect that the different range of fitness effects of the two types of mutations would have on evolutionary trajectories.

Interactions between epigenetic and genetic mutations during adaptation

An epigenetic system of inheritance (say DNA methylation) on its own could be thought to act just like a genetic system, although one with a higher mutation rate than DNA. However, an epigenetic system never transmits information across generations alone. Instead, epigenetic information is always transmitted along with genetic information. Moreover, the epigenetic systems with transgenerational inheritance discussed above (DNA methylation, RNAi) act by silencing genes, a result that can also be achieved by mutations in DNA alone. Because of this, an interplay between epigenetic and genetic evolution could arise simply from the phenotype (here: a silenced gene) being accessible either by epigenetic or by genetic changes. If the phenotype that can be implemented by either genetic or epigenetic changes is under selection, a particular form of epistasis emerges between genetic and epigenetic loci: Once the phenotype has been implemented by genetic change, there is no selection pressure on epigenetic variation, and vice versa. Here, we use a concrete example to explore how such an interplay arises, and how it affects adaptation.

Epigenetic mutations and gene silencing: A case study

Gene duplication is regarded as one main mechanism for generating new genes, which can in turn lead to novel function evolving through sub- or neo-functionalization, and it is an important driver of gene and genome evolution [30]. Gene duplication leading to the evolution of novel function is well-studied in plant secondary metabolism [31, 32], where differences in development and defense compounds can have profound consequences in terms of pathogen-host coevolution and the generation of phenotypic diversity [33]. The fate of duplicated genes depends crucially on whether or not beneficial mutations can occur before one of the copies becomes a pseudogene by accumulating neutral mutations. There are several models for understanding how duplicate genes are retained, and how novel function subsequently evolves. One key model, the duplication–degeneration–complementation model [34], suggests that degenerative mutations in regulatory elements increases the chances that duplicate genes are preserved because ancestral gene expression patterns can be subdivided. This in turn means that both copies are exposed to natural selection for longer, so that rare mutations that may confer novel functions have time to arise.

To understand how the genetic and epigenetic mutation system contribute to adaptive dynamics, we consider gene dosage compensation following a duplication event, and use the example of gene silencing. However, the general mechanism of altering promoter activity or specificity also applies to cases of sub- or neo-functionalization of duplicate genes [31]. One way of silencing a gene is to prevent transcription by interfering with the binding of trans-regulatory factors. Silencing can be achieved in any way that prevents regulatory factors from binding to DNA (changing gene expression without complete silencing may involve keeping a subset of regulatory factors from binding). Two mechanisms that can do this are cytosine methylation of the regulatory region or accumulating point mutations that destroy transcription factor binding sites, or some combination of the two [35]. Although other factors influence gene expression as well, we focus on these two mechanisms to probe the interplay between epigenetic and genetic changes.

Following gene duplication, the first changes to appear in the regulatory region of the gene would most likely be methylation marks, simply because their rate of appearance is much higher than the point mutation rate of DNA. Since they reduce gene expression, these epigenetic marks would be under positive selection, and could be fixed in the population. Further methylation marks would appear until the gene is completely silenced. Is that the end of the story? Not at all, for point mutations of DNA in the regulatory region arise as well, although much more slowly due to their lower mutation rate. If the gene is already fully silenced due to the methylation marks, these changes do not affect gene expression further. Thus DNA mutations accumulate neutrally, until binding sites would no longer function. What is the selection pressure on the methylation marks in this new situation? Suppose a site that was previously methylated in one generation is unmethylated in the next generation due to one of the frequent spontaneous changes. Without the changes in DNA that accumulated neutrally while the gene was silenced by methylation, demethylation would be selected against. However, once changes in DNA have destroyed binding sites, demethylation no longer changes gene expression, since transcription factors can no longer bind due to mutations in DNA sequence. The loss of the methylation mark is thus evolutionary neutral because there is no change in phenotype or fitness associated with this loss. Further losses of methylation marks will then accumulate. In the long run, the stabilizing selection that maintains gene silencing rests entirely on the genetic changes. These genetic changes, however, occurred neutrally and after the phenotype had already been established and transmitted over several generations by epigenetic changes. The epigenetic changes themselves were lost again after the phenotype had been achieved via genetic changes: they served as a stepping-stone during the adaptive process.

A general model for the role of epigenetic mutations in adaptation

To understand the interplay of epigenetic and genetic changes more generally, we constructed a simple mathematical model where fitness increases can either be achieved via a mechanism with high mutation rate (epigenetic changes) or via a mechanism with low mutation rate (genetic changes), see Box 1. Genetic

Box 1

A mathematical model of population dynamics with both genetic and epigenetic mechanisms

1. We consider the evolutionary dynamics of a set of variables $g_1, g_2, \ldots, g_k$ describing the state of the genetic system (DNA sequence) and a second set of variables $e_1, e_2, \ldots, e_l$ describing an epigenetic system.
2. We take the mutation rate to be $\mu_g = 10^{-6}$ for each of the genetic variables per generation, and $\mu_e = 10^{-4}$ for each epigenetic variables. For simplicity we use binary variables and $k = l = 10$.
3. The genetic variables specify a phenotype described by some function $w_{genetic}(g_1, g_2, \ldots)$, quantifying for instance to what degree a given gene is silenced. Analogously, the epigenetic variables specify a value of the phenotype $w_{epigenetic}$ $(e_1, e_2, \ldots)$.
4. The fitness corresponding to a particular state of the genetic and epigenetic variables is determined by the system with maximum value, $w = \max(w_{genetic}, w_{epigenetic})$. This is the simplest implementation of the tenet that genetic changes can also achieve whatever adaptation epigenetic changes can achieve. Here we present two types of fitness landscapes: (i) The single-peak landscape, $w_{genetic} = 1.5$ if $g_1 = g_2 = \ldots = g_k = 1$ (on-peak) and $w_{genetic} = 0.1$ otherwise (off-peak), and similarly $w_{epigenetic} = 1.5$ on the peak and $w_{epigenetic} = 0.1$ away from it. (ii) The multi-peak landscape, where each of the fitness values $w_{genetic}(g_1, g_2, \ldots)$ is drawn independently for each configuration $g_1, g_2, \ldots$ from a Gaussian distribution of mean 1.1 and standard deviation 0.25 (and analogously for the epigenetic variables). The latter results in a rugged landscape with many peaks and valleys.
5. We numerically simulate the evolutionary dynamics using a standard Wright–Fischer dynamics [13, 37] using a population of $N = 1{,}000$ individuals, see Fig. 2. We also explored a wide range of different population sizes, mutation rates and fitness landscapes, finding qualitatively similar results.

variables (nucleotides at different loci) and epigenetic variables (e.g. methylation states at different chromosomal positions or other mechanisms) jointly determine fitness. The resulting fitness landscapes allow us to link the dynamics of multiple genetic and epigenetic variables within population dynamics. This goes beyond the model by Pal and Miklos [23], where the epigenetic variables were modeled implicitly via phenotypic plasticity, and the analysis by Day and Bonduriansky [8] based on a single genetic and a single epigenetic locus.

We begin with a fitness landscape with a single peak in the genetic variables and a single peak in the epigenetic variables. Figure 2A shows a typical evolutionary dynamics: epigenetic variables have higher mutation rates, are faster to explore their fitness landscape, and tend to locate the fitness peak before the genetic variables do (red arrow). In most cases, the population thus initially owes its increase in fitness to changes in the epigenetic variables. During the period when the adaptive phenotype is encoded by the epigenetic variables, selection acts on the epigenetic variables, whilst mutations in the genetic variables are neutral (red highlighted region). Eventually, mutations in the genetic variables result in a sequence that encodes a high-fitness phenotype. Once this happens, the high mutation rate of the epigenetic variables turns from an asset to a liability, and lineages that owe their adaptive phenotype to the epigenetic variables suffer a higher mutation load than lineages that owe their adaptive phenotype to the genetic variables. The higher mutation load means that the average fitness of a population whose adaptive phenotype is encoded by the epigenetic variables will be lower than the average fitness of the population whose adaptive phenotype is encoded by the genetic variables [36]. Soon the entire population consists of individuals near the peak in the genetic variables (blue arrow). A switch of selection pressure from one mutation system to another has occurred; the phenotype is now encoded by the genetic variables, whereas the epigenetic variables now evolve neutrally (blue highlighted region).

The switch of selection between mutation systems can occur repeatedly on fitness landscapes with multiple peaks rather than a single peak, allowing successive moves from peak to peak. Figure 2B shows a typical evolutionary dynamics on a multi-peak fitness landscape (see Box 1 for details). The epigenetic variables allow the population to adapt quickly by finding a local maximum (red arrows), and subsequently evolve under stabilizing selection (red highlighted region). The genetic variables evolve neutrally during this time, until they reach an equal or higher local fitness maximum, at which point a switch occurs (blue arrow) so that phenotype is now determined by the genetic variables (blue highlighted region). A second switch of selection pressure can occur when the now neutrally evolving epigenetic variables locate a peak of even higher fitness, and so on until the global maximum has been reached. Selection pressure acting alternately on two systems, such that one system can evolve neutrally during periods when the other is under selection, is an efficient means of exploring the fitness landscape, compared to the dynamics of a single system. This effect emerges when there are two independent ways of encoding a phenotype, here genetic and epigenetic variables. Depending on the fitness landscape, a speed up in adaptation of several orders of magnitude can occur relative to a system with a single mutation system. This is shown by the green line in Fig. 2B, where adaptation from the same starting point and on an identical fitness landscape is modeled in a population with only a single mutation system. The population becomes stranded on a lower-fitness local optimum because it cannot explore the fitness landscape further so long as the stabilizing selection persists.

Insights from the model: How epigenetic mutations can affect adaptation

Our model predicts different ways in which epigenetic mutations can affect adaptation. First, adaptive phenotypic

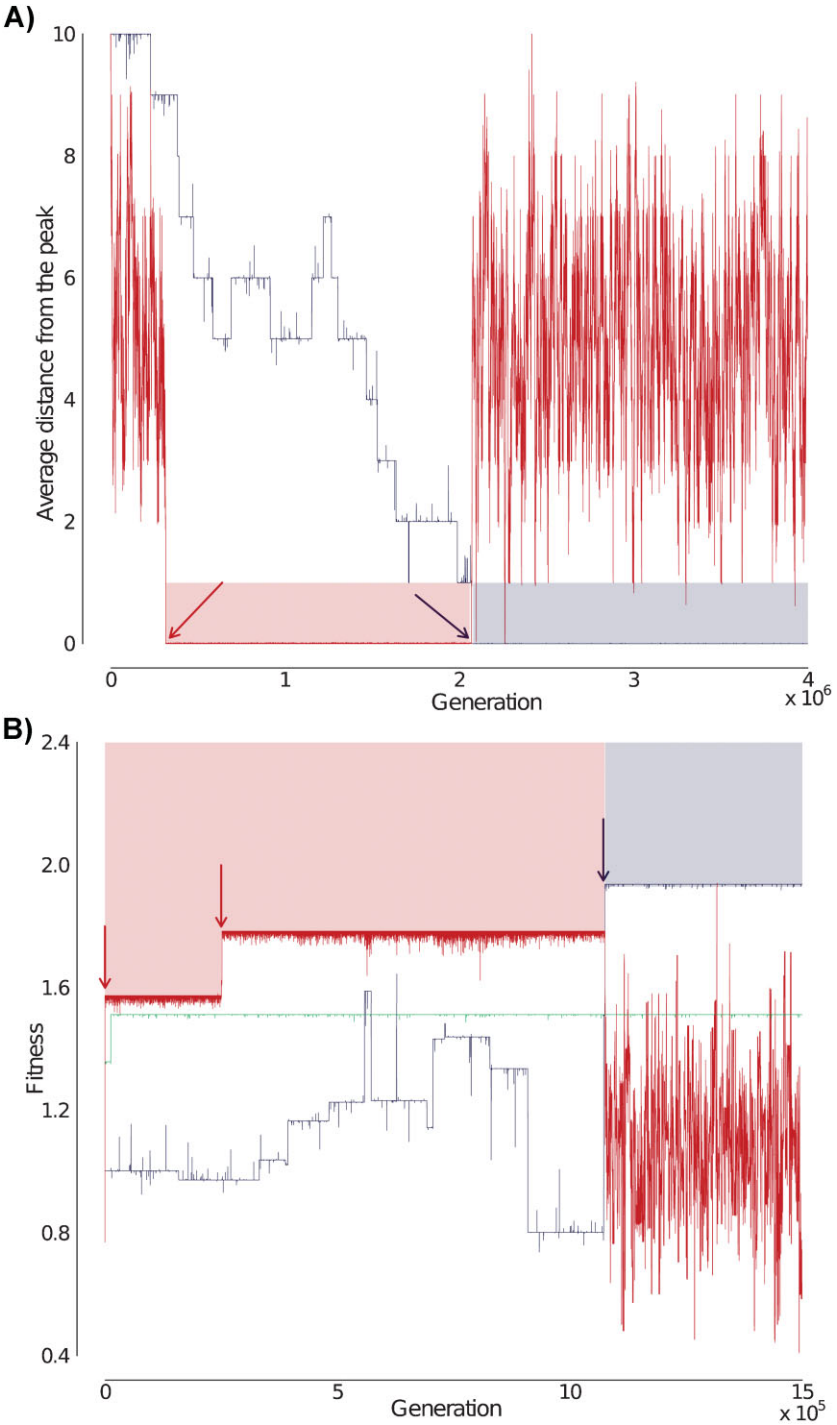

Figure 2. Evolutionary model dynamics using two mutation systems. The highlighted color regions indicate the mutation system which produces the fitter phenotype at a given time. **A:** Dynamics of adaptation on a fitness landscape with a single peak in the genetic variables and another peak in the epigenetic variables, see Box 1. We plot the population average of the distance of genetic variables from their peak (blue line) and analogously for the epigenetic variables (red line) on the *y*-axis and time (in generations) on the *x*-axis. Distance is measured by the minimal number of mutations required to reach the peak. The population is initially monomorphic. Epigenetic mutations occur quickly leading to an individual with epigenetic variables in the peak configuration (distance zero from the peak). This configuration is rapidly fixed in the population (red arrow). From this point on, the population has adapted to the single-peak landscape and phenotypic variation is minimized by stabilizing selection. Because only epigenetic variables are now under selection, genetic mutations can accumulate neutrally. Eventually, an individual with genetic variables in the peak configuration appears (blue arrow), after which stabilizing selection pressure switches to the genetic variables (blue highlighted region, see text). **B:** Dynamics of adaptation on a multi-peak fitness landscape. We plot the population average of the fitness associated with epigenetic variables ($w_{epigenetic}$) and genetic variables ($w_{genetic}$), respectively. Initial fitness increases are due to epigenetic mutations that are fixed in the population (red arrows), while genetic mutations accumulate neutrally, until they encode an even fitter phenotype (blue arrow). Now the epigenetic variables evolve neutrally, until they find a configuration with even higher fitness. The speed-up of adaptation compared to evolutionary dynamics with only a single mutation system is illustrated by simulating adaptation using genetic mutations only (green line), where the population gets stuck at a local maximum.

shifts can occur before adaptive genotypic change. Second, genetic variation is higher than expected during some part of adaptation because while an adapted phenotype is epigenetically encoded, genetic mutations can accumulate neutrally. Third, heritable epigenetic changes may allow mutation-limited populations to adapt by effectively increasing their mutational supply since encoding an adaptive phenotype epigenetically will keep population size up. We examine each of these in turn below. Previous work has largely focused on cases where no genetic variation exists, or on the case of a single allelic substitution at genetic and epigenetic loci, and focus on how epigenetic variation [8] or phenotypic plasticity modulated through epigenetic mechanisms [19, 23] affect population equilibria, such as levels of polymorphism. In contrast, our model shows possible effects of pure epigenetic variation on the dynamics of evolutionary trajectories involving substitutions at multiple genetic and epigenetic loci, and considers sequential fitness increases

during the exploration of single- and multi-peaked fitness landscapes.

When adaptation proceeds via epigenetic changes, the timing of adaptive genetic changes is affected. Specifically, when adaptation initially uses epigenetic mutations, fitness increases are decoupled from changes in genetic sequence, and the adaptive phenotype appears before the adaptive genotype. Estimates based on genetic information will underestimate the age of an adaptive event and make it difficult to reconcile the evolutionary histories of populations with the timing of environmental changes that impose selection. This dynamic of adaptive evolution is consistent with a ''phenotype first'' model [38, 39]. This is especially relevant to cases where we wish to understand how and if populations are able to adapt to current environmental change by reconstructing how populations have responded to analogous changes in the past, for instance climatic change. It also may be relevant for understanding the dynamics of changes in gene expression following duplication, as in our example.

A second effect of early adaptation via epigenetic mutations is a lack (or reduction) of selective constraints on genetic variables while selection pressure acts on the epigenetic variables. Since sequence changes accumulate neutrally during this period, we expect to see a higher level of genetic variance compared to a situation where adaptation is via genetic changes. Genetic sequence variation is neutral with respect to fitness during this time only because the epigenetic system is present and not because adaptive genetic mutations do not exist – genetic change could just as well generate the same fitness gain in the absence of epigenetic mutations, it would simply take longer on average to do so. As far as we know, this ''stepping stone'' effect is a novel expected effect of pure epigenetic variation on the dynamics of adaptive walks.

Finally, adaptive epigenetic mutations may allow a population to respond to environmental change even in the absence of standing genetic variation or the ability to generate it rapidly, and even in cases where no genetically encoded adaptive phenotypic plasticity exists. This is because epigenetic mutations can generate phenotypic variation in the absence of genetic variation [26], which can increase the chance that small populations persist in the face of environmental insult, either by using epigenetic change to adapt entirely or, to ''buy time'' to generate genetic mutations. This has the same end effect as adaptive phenotypic plasticity on probabilities of genetic adaptation, where populations with adaptive plastic responses to environmental challenges are more likely to withstand environmental insult [27]. Interestingly, more plastic populations of marine picoplankton are also predicted to be more likely to take advantage of nutrient enrichment such as increases in carbon dioxide [40], suggesting that not only are more plastic populations likely to persist in degrading environments, but are also more likely to thrive when environments improve. The effect of epigenetic mutations, however, does not require that the epigenetic marks be part of a genetically encoded adaptive plastic response, or that epigenetic mutations be directed in any way, just that some subset of the epigenetic mutations that occur are beneficial and heritable.

Conclusions and outlook

Our model shows that epigenetic mutations have the potential to affect both the tempo and outcome of adaptation. Testing experimentally if and how epigenetic mutations influence the genetics of adaptation remains a challenge. A system must be used where both very short and very long timescales can be readily observed. In addition, in order to observe the dynamics of adaptation, populations must be followed at least until there is evidence of novel genetic mutations rising in frequency in populations – often dozens or hundreds of generations. Because of this, microbial experimental evolution [41] using organisms with available reference genomes may be the best way to test our hypotheses. If epigenetic mutations contribute to microevolution, they must be characterized in the same way genetic mutations are. Specifically, estimates of epigenetic mutation rates and the distribution of fitness effects of epigenetic mutations are needed. Using mutation traps and whole epigenome sequencing could calculate epigenetic mutation rates in the same way as genomic mutation rates are now measured in microorganisms with reference genomes [25]. Taking the example of *Chlamydomonas reinhardtii* from the Ness et al. study [25], many replicate populations could be easily maintained, and since *Chlamydomonas* divides more than twice a day, the stability of epigenetic mutations could be measured by sequencing the same populations at several timepoints. Because *C. reinhardtii* has relatively low methylation levels [42], there should be a high signal-to-noise ratio in this system, as well as a reasonable chance that epigenetic mutations have a phenotypic effect. Once epigenetic mutants had been found in this system, it would also be easy to measure the distribution of effects of epigenetic mutations by comparing the growth rates of epimutants to lineages with the same genotype and a reference epigenetic pattern.

While it is uncontroversial that long-term evolution involves genetic change, we have no idea what proportion of phenotypic adaptation relies immediately on genetic variation. As more and more genetic data become available, a major challenge will be to correctly interpret what genetic variation alone tells us about adaptation. To do this, we must know the relative contributions of genetic and epigenetic mutations to adaptation. More generally, the debate on the role of ''soft'' inheritance in evolution has been revived in the light of a growing understanding of how non-genetic inheritance functions [10], and a growing body of theory is starting to provide tools to investigate how non-genetic inheritance affects evolution. Our simulations expand this body of theory by showing how epigenetic mutations can affect adaptation itself as well as the patterns of genetic variation that we use to infer the timing and magnitude of adaptive events during adaptive walks.

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