The role of epigenetics in plant adaptation

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Abstract

Recent work in the field of plant epigenetics is adding to a growing understanding of how epigenetic variation can be an important source of phenotypic variation in natural populations. Therefore, it has the potential to play a major role in adaptation to environmental change. Most epigenetic variation is reset between generations, however, in some instances environmentally-induced epigenetic variation can result in heritable phenotypic plasticity that invokes Lamarkian-like inheritance. Epigenetic variation can also be the result of random epimutations that can have both higher mutation and reversal rates than DNA sequence mutations. We discuss several examples documenting epigenetic variation in wild populations. We also discuss laboratory studies that investigate the rate of epimutations and reversals, and how that has been incorporated into evolutionary theory. We suggest that modern evolutionary theory will benefit from the incorporation of epigenetics, but it is not in need of a complete revision, as has been suggested.

Epigenetics in ecology and evolution

There has been long-standing evidence of transgenerational epigenetic inheritance, such as paramutation in maize[1] and imprinting in mammals,[2] but the general biological community did not take notice until it became abundantly clear that it was a widespread phenomenon in plants and animals, and not limited to a few very specific examples. The fact that environmental cues in one generation can cause epigenetic changes that are inherited for multiple generations, which has been referred to as Lamarkian inheritance or inheritance of acquired characteristics,[3,4] has particularly intrigued evolutionary biologists.

Epigenetics involves meiotically and mitotically stable alterations in gene expression that are not based on DNA sequence changes, but involve processes that impact the packaging of DNA (chromatin structure).[5] These processes include the addition of methyl groups to the fifth carbon in cytosine molecules (DNA methylation), and modification of histones may be influenced by transposable elements, which are often methylated, and small RNAs which can direct DNA methylation and chromatin remodeling at their target loci.[6-9] Chromatin structure then alters the availability of DNA to transcription factors, and influences whether genes can be expressed.[10] Although believed to have evolved in part to protect against genome perturbations, such as transposable elements and retroviruses,[11] epigenetic processes play a crucial role in cell differentiation and development, and are probably responsible for many aspects of behavior and phenotypic plasticity.[12]

Epimutations can create heritable epialleles, the epigenetic equivalent of genetic alleles. They may be caused by errors in methylation maintenance,[13,14] *de novo* methylation,[15] or other chromatin remodeling factors,[10] or they may be triggered by a particular environmental stimulus, creating a type of transgenerational plasticity.[16] Although epigenetic variation can occur in the absence of genetic variation, genetic variation can influence epigenetic variation and the epimutation rate in a number of ways. For instance, variation in the presence of cytosines that can be methylated,[17] transposable elements,[18-21] small RNA production,[22] and genes controlling histone modifications and chromatin structure[10] can all influence whether a gene is subject to epigenetic silencing. Thus, selection on the epigenotype may act directly on transgenerationally heritable epialleles, or it may proceed by selection on DNA polymorphisms that influence epigenetic state. Epigenetic variation can be a significant source of natural phenotypic variation; therefore, it has the potential to play a major role in adaptation to environmental change. A simple hypothetical scenario may illustrate the possibility that adaptive phenotypic evolution may occur via epigenetic modification even though the population is genetically homogeneous (Figure 1).

This example illustrates how environmentally-induced phenotypic change may be mediated via epigenetic mechanisms. In this case, nutrient stress could cause phenotypic variation within an otherwise genetically homogeneous population. There are various reasons why some plants may change phenotypes while others do not. For instance, there may be some stochasticity such that the methylation probability of a particular region given the environmental cue may be less than 1, there may be micro-heterogeneity in the environmental cue, or there may be genetic differences among individuals that affect the availability of sites that can be methylated within a particular region. Although the phenotypic change is not necessarily adaptive in the environment that cues it, the induced phenotypic variation (*e.g.* a smaller flower size in some plants) may offer the opportunity for the selection to act. In this example, small flowers may require fewer resources and result in higher seed maturation and therefore higher fitness. If the change in methylation is transgenerationally inherited (transgenerational plasticity), the new epimutation can spread into the population, and adaptive evolution can occur even in the absence of genetic change.[23] Alternatively, even if the methylation change is not inherited, but there is genetic variation in the ability to be cued by the environment (plasticity), selection can act on this genetic variation.

Modern evolutionary theory is primarily based on the inheritance of random genetic variation, so there has been ample discussion whether evolutionary theory requires revision in light of epigenetics.[4,24-27] In order to assess the importance of epigenetics in evolutionary processes, it is first necessary to show that epigenetic variation exists in wild populations, and second that this variation correlates with phenotypic variance that is subject to selection. Next, it is necessary to determine what epigenetic variation is transgenerationally inherited. In this review, we first describe examples of naturally-occurring epigenetic variation that influences plant reproduction,

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followed by recent evidence for transgenerational plasticity in response to stress. Next, we discuss studies of DNA methylation variation in wild populations, followed by an overview of recent laboratory experiments in which heritability of methylation variation is directly analyzed. We conclude with a discussion on how epigenetics fits into post-Modern Synthesis evolutionary theory from both a mechanistic and theoretical viewpoint.

Natural epigenetic variation and reproduction

Research on epiallelic variation traces its roots to a seminal paper showing that the first natural morphological mutant described by Linnaeus is actually a transgenerationally heritable epimutation, caused by hypermethylation, and not by a DNA mutation.[28] *Linaria vulgaris* flowers are typically bilaterally symmetrical and bee pollinated.[29] The epimutation suppresses transcription of the *Linaria-like-CYCLOIDEA (Lcyc)* gene in developing flowers, causing them to become radially-symmetrical,[28] and not likely to be effectively pollinated by bees. Shifts from bilateral to radial symmetry are often associated with a change in pollination syndrome.[30] Another spontaneous, heritable epimutation, caused by hypermethylation in the promoter-region of the *COLORLESS NON_RIPENING (CNR)* locus of tomatoes, is thought to cause non-ripening fruits,[31] and is perhaps regulated by small, non-coding RNAs.[32] Although this study was in cultivated tomato, it demonstrates the impact of natural epigenetic variation on fruit color and ripening characteristics, which play a central role in seed dispersal.

In *Arabidopsis thaliana*, two highly studied genes influencing the timing of flowering are regulated, at least in part, by epigenetic mechanisms. In *Arabidopsis, FLOWEIRING LOCUS (C) (FLC)* is an important gene for synchronizing floral timing with seasonal cues. Specifically, FLC suppresses flowering until a sufficiently long cold period has been experienced (vernalization), so that plants know when to time flowering in spring.[33] Vernalization causes epigenetic changes in the chromatin structure of FLC, suppressing FLC expression, and permitting flowering.[34] There is variation among ecotypes in the genes that control *FLC* chromatin structure, and therefore epigenetic variation at the *FLC* locus among ecotypes, which results in variation in flowering time.[22,35,36] Further, there are associations between variation in these genes and latitude, winter temperatures, and precipitation,[35,36] suggesting that their influence on the epigenetic control of *FLC* could be important for adaptation to seasonal environments associated with local climates.[35] Epigenetic variation in the *FLOWERING WAGENINGEN (FWA)* gene can also influence flowering time. FWA is expressed only in the endosperm of wild-type A. thaliana, but heritable[37] lab-induced epialleles cause FWA to be expressed in vegetative tissue, producing a late-flowering phenotype.[38] These epialleles are independent of DNA variation. There is natural variation within and among other *Arabidopsis* species in both the level of *FWA* promoter methylation and level of vegetative expression, which may be caused by DNA variation in the FWA promoter.[39] This natural variation outside of *A. thaliana* does not appear to influence flowering time,[40] however there may be an effect on other phenotypes, such as endosperm development. Further, the fact that *FWA* epialleles occur in the lab shows they are possible and may occur in nature. These examples give weight to the argument that epigenetics could result in ecologically-important phenotypic variation. While the heritable *Lcyc* epiallele in *Linaria vulgaris* and CNR epiallele in tomatoes appear to be entirely epigenetic, and do not appear to be linked to DNA variation, the natural epigenetic variation in Arabidopsis flowering time appears to be controlled, at least in part, by DNA sequence variation. Thus, selection on epigenetic variation may act either directly on the epigenotype or on DNA variation that influences the epigenotype.

Transgenerational plasticity in response to stress

Epigenetic mechanisms can play an important role in plastic responses to the environment[34] and have been particularly studied in relation to plant stress responses. As sessile organisms, plants often display high levels of phenotypic plasticity to cope with stress. Priming, an effect in which stress exposure causes a plant to either exhibit higher resistance or faster response to that stress in the future, can, in certain examples, be linked to epigenetic marks that activate transcription of stress-related gene pathways.[41] In some cases, *stress memory* has been shown to pass from parental generations to unstressed offspring,[41] presumably to prepare offspring for an environment containing the same stressors.[6]

Two ground-breaking studies have linked epigenetic variation to the transmission of stressed phenotypes from the parental generation to unstressed offspring. In *Mimulus guttatus*, simulated herbivory (leaf damage) induced trichome production on the underside of leaves, a well-known response to deter future herbivory. The response was linked to the epigenetic down-regulation of a specific candidate gene (*MgMYBML8*). This epimutation was inherited by unstressed offspring that also displayed increased trichome production when compared to control plants.[42] In a different approach, genome-wide DNA methylation

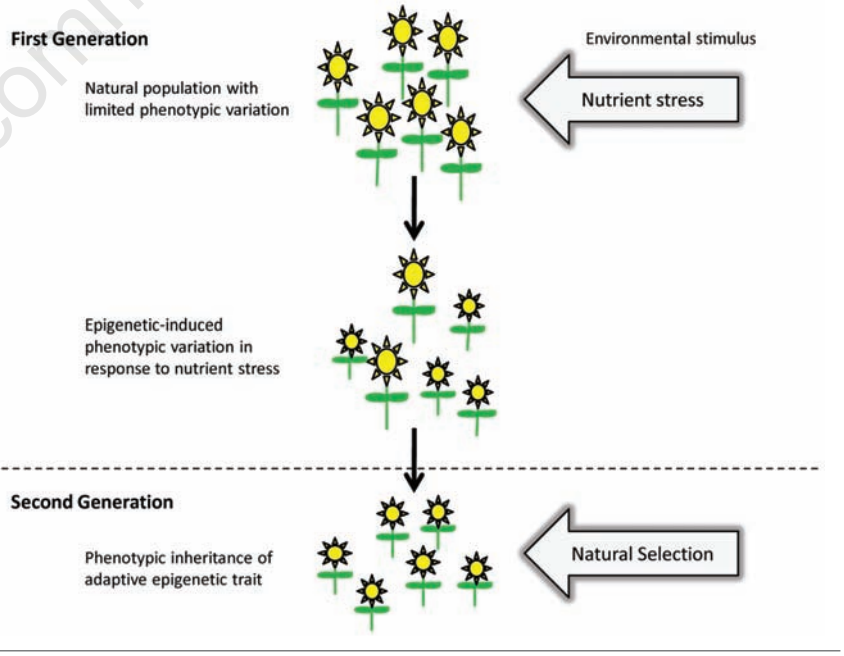

Figure 1. A hypothetical example of epigenetic-induced phenotypic variation in response to an environmental cue. In the parental generation, a natural plant population is exposed to nutrient stress which induces phenotypic plasticity that is of epigenetic origin. The new phenotypic mean is skewed towards smaller phenotypes which require lower nutrient levels to successfully reproduce. The adaptive phenotypic trait is inherited by the offspring, as are the associated epigenetic markers.

profiles were compared between control individuals of apomictic dandelions (*Taraxacum officinale*) and those exposed to chemical simulation of herbivore or pathogen attack. Significant genome-wide methylation changes were observed in stressed plants which displayed stunted phenotypes; the stressed phenotype was inherited for three generations as were most of the methylation changes.[43] The genetic uniformity of asexual plants makes this an ideal system for demonstrating the impact of environmental cues on epigenetic inheritance.[43]

These two studies are among the first to document transgenerational plasticity in plants that is directly correlated to epigenetic modifications, although it has long been speculated. Other noteworthy studies have linked ecologically-important epigenetic responses to stress factors, although transgenerational inheritance was either unexplored or has been unapparent. These include global hypomethylation in hemp (*Cannabis sativa*) that is exposed to heavy metals,[44] drought-induced methylation changes in rice, *Oryza sativa*, that may increase drought tolerance,[45] and transcription activation of repetitive elements due to chromatin modification in *Arabidopsis thaliana* that is exposed to prolonged heat stress.[46] Activation of repetitive elements in response to stress is extremely interesting, since this is likely to increase the mutation rate and increase phenotypic variation, potentially increasing the chance that a stress-adapted mutant will arise.[47]

In an extreme example of environmentally-induced plasticity, exposure to acute salt stress in the salt-tolerant plant, *Mesembryanthemum crystallinum,* resulted in the methylation-directed down-regulation of loci responsible for switching from the C3 photosynthetic pathway to crassulacean acid metabolism (CAM) pathway.[48,49] Even if not transgenerationally inherited, these transient stress responses can increase fitness while avoiding the cost of constitutive expression of stress-related genes.[41] A great deal of additional research is needed to determine how frequently stress-response traits, as well as other phenotypic traits, can be transgenerationally inherited.

Methylation variation in natural populations

In order to understand the role of epigenetic effects on plant adaptation, it is necessary to understand the occurrence and structure of epigenetic variation in nature.[26,50] To date, there have been only a few studies on natural populations, however, tools borrowed from early DNA sequence variation analysis, such as amplified fragment length polymorphisms (AFLPs) modified to detect differences in cytosine methylation (methylation-sensitive AFLPs; MSAP), have allowed the quantification of epigenetic variation to reach beyond the laboratory and model organisms.

In one study using this technique, Herrera and Bazaga[51] showed that both MSAP epigenotypes and AFLP genotypes of individuals are correlated with long-term herbivory levels in natural populations of the wild violet (*Viola cazorlensis*). They identified six AFLP *loci* related to 44% of variation in herbivory, and showed that the epigenotype was significantly correlated with genotype at these six herbivory-related AFLP loci.[51] It is difficult to make strong conclusions about causal relationships, however, as the differences among epigenotypes could be caused by variation in herbivory, or the differences in herbivory (herbivore resistance) could be caused by variation among epigenotypes. Methods such as common-garden experiments that control the environment, or studies of genetically uniform plants are necessary to distinguish among environmental, epigenetic, and genetic sources of variance.[6] Nonetheless, this study clearly shows the importance of the interplay of epigenetics and genetics in herbivory dynamics in a natural population.

Several recent studies have applied the MSAP technique to compare global methylation patterns among individuals collected from contrasting environments. A surprising consistency in findings has emerged from these early population-level studies. First, levels of genome-wide epigenetic variation are higher than genetic variation, even when the epigenotype was scored in a single tissue and single developmental stage.[51-55] Second, among-population epigenetic variation is higher than within-population variation, even when there is no overall genetic differentiation among populations.[54,55] Further, epigenetic variation is highly correlated with environment, both within and among populations.[9, 51,54-57] This may be due to environmental influences on the epigenetic state (plasticity), but could also be due to selection on the epigenotype, or on genes influencing the epigenotype. These findings are being interpreted as evidence that epigenetic mechanisms are important for responding to the environment, and that they may contribute to adaptive divergence among populations.[7,58]

Another source of natural epigenetic variation arises from the processes of polyploidization and hybridization, a common phenomenon believed to be in part responsible for the extreme levels of species diversity in plants.[59] Genome-wide epigenetic changes are induced by genome duplication events and are believed to be a coping mechanism for the genome shock caused by these processes.[19] Moreover, the novel epigenetic variants produced by genome duplication provide the potential for phenotypic and ecological divergence between polyploids and their parental taxa,[60] or among sister polyploid taxa that have arisen from the same parental taxa.[59,61,62] MSAP comparisons among three sister allopolyploid species of the orchid, *Dactylorhiza*, growing in three different environments, showed a striking divergence in methylation profiles that were highly correlated to growing environment.[61]

The examples included in the section highlight an emerging and rapidly growing field of *population epigenetics* but they also reflect some of the challenges. Studies on natural populations to date have only speculated about transgenerational inheritance of the observed epigenetic variation, and are complicated by the correlation between genetic and epigenetic variance. Further, it is typically not determined whether the observed variation in DNA methylation has any functional consequences.[9] Despite these obstacles, these studies are leading the way forward to a better understanding of how epigenetic processes contribute to adaptation to local environments and their role in adaptive divergence.

Methylation variation in Recombinant Inbred Lines

Unlike the studies on natural populations discussed above, laboratory populations have been used to directly study the inheritance of methylation polymorphisms, and their link to phenotypic variation. By creating highly inbred lines that are virtually genetically identical but have introduced epi-mutations, epigenetic Recombinant Inbred Lines (epiRILS) have been used to decouple the effects of the genotype and epigenotype as sources of trait inheritance.[63] Two groups developed isogenic lines of *Arabidopsis thaliana*, both bred from a wild type parent and a parent with a single loss-of-function mutation in a gene associated with methylation control, *MET1*[64] and *DDM1*;[37] thus both of these studies eliminated genetic variation and exaggerated epigenetic variation.

The first major finding from both studies is that extensive epigenetic variation not only differed greatly from the parents, but it persisted over at least 8 generations in the absence of selection.[37] Second, this epigenetic variation resulted in increased phenotypic variation in ecologically-important traits such as flowering time,[37,64] and traits that can influence plant fitness, such as plant height[37] and biomass.[64]

Within the epiRIL populations, the vast majority (70%) of methylation changes reverted to the wild-type state within eight generations.[37,64] This has been interpreted as evidence of the instability of epialleles and of a genomic *rescue system* to maintain genomic

integrity.[65] Interestingly, broad-sense heritability estimates derived from these epiRIL populations is similar to heritability for many quantitative traits presumed to have a genetic basis.[37,66] These exaggerated *MET1/DDM1* loss of function mutants are not likely reflective of natural populations, however, and there is clearly a need for this type of study on natural ecotypes. Nonetheless, these epiRILs show that methylation variation is a trangenerational source of phenotypic variation, and offer some insight into how epialleles contribute to the heritability of complex quantitative traits. Additionally, these papers suggest how it may be possible to map variation in cytosine methylation to disentangle the genetic and epigenetic contributions to natural variation in quantitative traits, and identify the functional consequences of variation in DNA methylation.

Epimutations and evolutionary theory

Modern evolutionary theory is generally based on a strict definition of inheritance of random genetic variation. Because epigenetic variation can play a role in inheritance, it is necessary to consider how it should be incorporated into evolutionary theory and population genetics. Although some researchers have even suggested that a complete revision of evolutionary and population genetics theory is needed,[4,25,27] we believe that epigenetics can be incorporated into existing theory with some simple modifications. First, random epimutations, which are not induced by the environment, can be treated very much like random genetic mutations, with minor modifications to theory. Second, some epimutations are very different from traditional genetic mutations because they are influenced by the environment. These environmentally-cued epimutations, which are also referred to as transgenerational plasticity, can be modeled much like adaptive plasticity or adaptive maternal effects, which have been relatively well studied.[67-69] In this section, we discuss data on several features of random epimutations, relevant to evolutionary theory, and some approaches that have been used to model random and environmentally-cued epimutations.

The rate of natural random epimutation and the stability of epialleles is not yet well understood, however this is a critical factor for incorporating epigenetics into evolutionary theory. The epimutation rate is likely to influence epigenetic diversity, equilibrium frequencies of epialleles, and therefore how random epigenetic variation will contribute to adaptation. One detailed study in *Arabidopsis thaliana* makes great strides towards understanding the rate of natural, spontaneous, random epimutations in a single growth environment. Becker *et al.*[70] compared genome-wide variation in DNA methylation among 10 *Arabidopsis thaliana* lines that were derived from a common ancestor 30 generations ago. The epimutation rate for single cytosines was far higher than the genetic mutation rate. However, the epimutation rate of larger, contiguous regions of methylation, which are more likely to have functional consequences, was similar to the genetic mutation rate. Further, the methylation status of certain sites was highly mutable while other sites were stable. Thus, epimutation has the potential to occur at rate much higher than the mutation rate, at least at some sites. Although this study investigated the natural epimutation rate in plants that were not subject to demethylating agents such as 5-azacytidine, the study was conducted in the lab. Epimutation rates in natural populations could be influenced by the environment, and could be quite different. Thus similar studies in more natural environments will be valuable. Research is also needed to understand how these changes in cytosine methylation correspond to phenotypic changes, and to measure the epimutation rate for phenotypic traits.

Another important empirical observation is that reverse epimutations are much more common than reverse nucleotide mutations.[70] This is due to the high epimutation rate at some sites and the fact that an individual cytosine has only two possible states (methylated or unmethylated), whereas nucleotide sites can have four different states. The rate of reversals is important for the incorporation of epigenetics into population genetics models. Further, frequent reversals facilitate switching back and forth between two phenotypes, which may be beneficial if the environment fluctuates between two different states.

Similar to nucleotide mutations, epimutations have the potential to be beneficial, neutral or deleterious. In a stable environment, where most individuals are well-adapted, mutations or epimutations are likely to reduce an individual's fitness, creating genetic or epigenetic load. Stenøien and Pederson[71] modeled the negative effects of epigenetic load. They show that the fitness consequences of epimutations are analogous to the effects of deleterious genetic mutations, and load is primarily determined by the epimutation rate and degree of reversibility. Since the heritable epimutation rate may be quite high relative to the mutation rate, epimutation has the potential to increase load considerably. Even epimutations that cannot be transgenerationally inherited have the potential to considerably decrease fitness. They suggest that these epimutations are similar to somatic mutations, and because the epimuation rate can be orders of magnitude higher than the somatic mutation rate, especially as individuals age, epigenetic load will be much more severe than somatic genetic load. However, to understand the impact of epigenetic load relative to genetic load, we need better estimates of the fitness consequences of both heritable and non-heritable epimutations.

In contrast to a stable environment, mutations or random epimutations may be beneficial in a temporally or spatially variable environment. If there are two environments, and two heritable epialleles, where one has higher fitness in each environment, a high rate of environmental change favors a high rate of epimutation.[72-74] Epimutations in some fraction of the progeny allow an individual to produce offspring with a mix of phenotypes in the face of unpredictable environmental fluctuations from one generation to the next. The probability of each phenotype should be determined by the probability of being subject to selection in each environment.[73] This is basically a bet-hedging strategy.[75] Since epigenetic mechanisms are more likely to permit frequent switching between two allelic states than genetic mechanisms,[70] epigenetic mechanisms may be favored for traits that influence survival in a variable environment.[73]

Models have also investigated the adaptive significance of heritable, environmentally-cued epimutations (transgerational plasticity) vs. a purely genetic strategy of phenotype determination or a purely plastic strategy (environmentally-cued, but not transgenerational).[74] Jablonka *et al*. (1995) suggests that transgenerational plasticity is an intermediate strategy between plastic and genetic strategies. On the other hand, Shea (2011) views transgenerational plasticity as being identical to adaptive maternal effects. Like the models of random epimutation, these models also focus on environmental variation, and one advantage of transgenerational plasticity could be the production of offspring with a mix of phenotypes in the face of an unpredictable environment. The frequency of each phenotype should be determined by the probability of being exposed to selection in each environment,[74] The pattern and frequency of environmental change is likely to determine when transgenerational plasticity is beneficial.[67, 73,74] If the environment changes frequently within a generation, there would seem to be no benefit to transgenerational plasticity. Likewise, if it remains stable for hundreds of generations, selection would likely fix a single genetically-determined phenotype before the environment changed. Yet if it remains stable for a few generations so that the parent's environment predicts the offspring's environment with some accuracy, it may be beneficial to inherit the parent's phenotype rather than relying on an environmental cue to direct development.[73] This inheritance of cues from the parental environment may be especially

beneficial if there is some time lag between detection of the environmental cue, and assumption of the appropriate phenotype.[73] Similarly, transgenerational plasticity could be beneficial because the parent can detect the environmental cue more reliably than the offspring.[67] For instance, if the parent experiences herbivory, and herbivore abundance cycles with a period of several years, it is likely that her offspring will experience the same herbivory. Offspring may then benefit by producing defenses such as trichomes[42,76-78] or glucosinolates[77] in anticipation of herbivory. Similarly, if the parent does not experience herbivory, there it is likely that offspring will not either, and they can avoid the costs of producing defenses.

Considerable progress has been made in incorporating epigenetics into evolutionary theory, however many avenues of research remain yet to be explored. For example, further research is needed to understand why some sites are highly epi-mutable, but others are more stable. Is the explanation purely mechanistic, reflecting different mechanisms of methylation maintenance, or has selection shaped the epimutation rate just as it has shaped the mutation rate? Are the more stable epimutations more likely to have functional consequences? Perhaps, like non-synonymous DNA sites, the stable sites are subject to purifying selection, while methylation at unstable sites have no phenotypic consequence and are therefore neutral with respect to selection, similar to synonymous DNA sites. Additionally, at a small number of sites, a high epimutation rate could be beneficial, and therefore positively selected. We still know very little about natural epimutation rates at the phenotypic level and the transgenerational stability of epimutations that influence phenotype. These epimutations are far more likely to be subject to selection, and have more potential to contribute to adaptive evolution. Other unanswered questions include: Are random epimutations more stable than environmentally-cued epimutations? How many generations does an environmentally-cued epimutation persist in a non-matching environment? What conditions would selectively favor the maintenance of an environmentally-cued phenotype for multiple generations in a non-matching environment?

Concluding thoughts

Much remains to be explored in the field of epigenetics, both mechanistically and ecologically before the true impact of epigenetics on plant adaptation is understood. It is clear however, that both heritable and non-heritable epigenetic variation is an important source of variance in ecologically important traits such as reproduction and stress tolerance. Epigenetic differences between contrasting habitats are further evidence that epigenetic mechanisms are important in plant responses to the environment in natural populations. This variation can result in environmentally-induced phenotypic plasticity, which may be transgenerationally inherited, although there are currently only a few good examples of epigenetically-induced transgenerational plasticity. Nonetheless, studies in natural environments demonstrate that epigenetics are important for adaptation to environmental change. Epigenetic variation may be controlled by environmental variation and/or genetic variation, or it may be independent of both. Thus selection may influence epigenetic traits either through selection on the genes that control epigenetic variation or on heritable epialleles. Future research efforts to untangle the sources of epigenetic variation within specific pathways or systems will be necessary to better understand genotype by epigenotype by environment interactions and how they relate to selection. Epigenetic variation can contribute to a large fraction of phenotypic variance, and may be especially important in populations with little genetic variance, or in habitats exposed to rapid environmental change. Research addressing the level of heritable and non-heritable phenotypic variation caused by epigenetic variation in populations with low genetic diversity will be especially useful.

The rapid pace of advancement, coupled with increased affordability, of next generation sequencing technology will allow for more comprehensive studies on genome-wide epigenetic variation in non-model organisms and natural populations. For example, whole-genome bisulfite treatment of DNA, or chromatin immunoprecipitation, followed by next generation sequencing provides genome-wide information on site-specific methylated sites or histone modifications, respectively.[79] The next step in epigenetics research is to link gene expression levels to observed epigenetic variation. Entire transcriptomes (including those for small RNAs) can now be obtained in a few days, allowing for direct comparisons in expression levels between contrasting environments. Most notably, these methods do not require an annotated genome. At this level, it will be easier to connect variation in DNA methylation or other epigenetic marks to phenotypic and environmental variation. Linking epigenetic variation to differential gene expression is the next step in epigenetics research. Quantitative trait loci mapping and association studies are needed to solidify the relationship between the epigenotype, genotype, and phenotype. To understand the role of epigenetics in plant adaptation, it will take the collaboration of molecular biologists and evolutionary ecologists to combine mechanistic information into population genetics models and ecological theory. The rapid pace of advancement in the field of epigenetics will continue to shape our understanding of the mechanisms controlling and creating phenotypic variation, and its implications for evolution.

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