Plant Transgenerational Epigenetics

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Abstract

Transgenerational epigenetics is defined in opposition to developmental epigenetics and implies an absence of resetting of epigenetic states between generations. Unlike mammals, plants appear to be particularly prone to this type of inheritance. In this review, we summarize our knowledge about transgenerational epigenetics in plants, which entails heritable changes in DNA methylation. We emphasize the role of transposable elements and other repeat sequences in the creation of epimutable alleles. We also argue that because reprogramming of DNA methylation across generations seems limited in plants, the inheritance of DNA methylation defects results from the failure to reinforce rather than reset this modification during sexual reproduction. We compare genome-wide assessments of heritable DNA methylation variation and its phenotypic impact in natural populations to those made using near-isogenic populations derived from crosses between parents with experimentally induced DNA methylation differences. Finally, we question the role of the environment in inducing transgenerational epigenetic variation and briefly present theoretical models under which epimutability is expected to be selected for.

INTRODUCTION

The term epigenetics was coined in 1942 by the geneticist and developmental biologist Conrad H. Waddington to advocate the need to understand, in the framework of the theory of epigenesis, the mechanisms by which genes (the basic subject matter of genetics) bring about phenotypic effects (145). However, developmental genetics as a discipline came only much later, and the term epigenetics was not much used until Robin Holliday proposed to designate by it the study of changes in gene expression that are heritable (through mitosis, as in development or cancer, or through meiosis) but not due to changes in DNA sequence (50). This new definition stemmed mainly from both empirical and theoretical work on the inheritance of DNA methylation across cell divisions, which could explain stable patterns of gene expression. So defined, epigenetics gained immediate popularity, in great part because it also provided an umbrella term for the numerous phenomena and processes that do not conform to Mendel's laws, like X-chromosome inactivation, parental imprinting, or paramutation (8). Since then, the word epigenetics has acquired numerous and sometimes widely divergent additional meanings, notably following the realization that far from being a mere DNA packing device, chromatin is a highly dynamic structure that plays a pivotal role in the regulation of gene expression (10). As the title of this review implies, it is Holliday's definition of epigenetics that we use here. Nonetheless, true to Waddington's conception, epigenetics is classically envisaged in the context of development, and there are indeed multiple developmental processes in plants as well as animals that involve somatically heritable changes in gene expression.

Random X-chromosome inactivation in female mammals, which ensures dosage compensation between the two sexes, undeniably offers one of the most striking examples of a developmental epigenetic switch in eukaryotes. This process is triggered in each cell of the early embryo at random on one or the other of the two X chromosomes and leads to the stable shutting down, in a clonal manner and in all somatic lineages, of most of the genes carried by that chromosome (41). Another striking example is parental genomic imprinting, which affects over 100 loci in the mouse (20). Genomic imprinting is established in a locus-specific manner in either the male or the female germline and results in parent-of-origin-dependent gene expression in the progeny. By definition, both X inactivation and genomic imprinting must be reset at every generation and as was initially hypothesized for X inactivation (115), the two processes rely at least in part on DNA methylation (20, 41).

Plants also exhibit genomic imprinting, but unlike in mammals, this process involves targeted DNA demethylation rather than methylation and typically does not require resetting (40). Indeed, genomic imprinting in plants is generally restricted to a single tissue, the endosperm, which feeds the embryo but does not otherwise contribute to it (40). Plants, being sessile organisms, absolutely need to align their development with environmental conditions, and a major role for epigenetics in this context has been identified in at least one case, vernalization. Specifically, in *Arabidopsis* accessions that overwinter, the floral repressor gene *FLOWERING LOCUS C* (*FLC*) becomes transcriptionally repressed during cold and remains epigenetically silenced during subsequent growth in warm conditions, thus allowing flowering (7). Importantly, this memory system is reset at every generation and does not depend on DNA methylation. Rather, stable repression of *FLC* is achieved through another evolutionarily conserved epigenetic mechanism, which involves Polycomb-group proteins and trimethylation of lysine 27 of histone H3 (H3K27me3) (7).

In contrast to developmental epigenetics, transgenerational epigenetics implies an absence of resetting of epigenetic states in the germline or the early embryo and may thus reflect failed reprogramming (48). In this review, we summarize our knowledge about transgenerational epigenetics in plants, which, as in mammals, entails heritable changes in DNA methylation but in which the largest number of cases has been reported. We emphasize the role of repeat sequences in creating epimutable alleles, and we argue that failed reinforcement rather than failed reprogramming

of DNA methylation over such sequences is responsible for the inheritance of variants in DNA methylation states.

NATURAL EPIALLELES

In the context of this review, epialleles are defined as the forms of a gene that are responsible for heritable phenotypic variation but do not entail a change in DNA sequence (either at the locus or elsewhere in the genome). Thus, there can be several epialleles for a given allele, and these will typically be caused by heritable changes in the regulation or level of gene expression. In fact, epialleles of protein coding genes may lead to distinct products only when alternative transcripts are formed. There are very few known cases of natural epialleles, and most have been found in plants (146), which suggests that compared to allelic variation, epiallelic variation is very rare in nature. However, as discussed below, the question of the relative contribution of epimutations and DNA mutations to natural phenotypic variation is not yet resolved.

The first natural plant phenotype for which the molecular basis could not be attributed to a change in the DNA sequence is the peloric variant of *Linaria vulgaris* (toadflax). Flowers of peloric plants have radial instead of bilateral symmetry, and genetic analysis indicates that the peloric phenotype is caused by variation at the *Lcyc* locus (26). However, no sequence polymorphisms specific to the mutant allele were found, which instead showed high DNA methylation and reduced expression compared to the wild type (26). Confirmation that the peloric variant analyzed was caused by an epimutation rather than a DNA mutation came from the observation of frequent somatic instability of the peloric phenotype, which is manifested by the appearance of stems with either semipeloric or almost wild-type flowers. Remarkably, there was a clear correlation between the intensity of the revertant phenotype and the degree of DNA methylation loss at the *Lcyc* locus, indicating that epiallelic variation at a locus can be continuous (26). Although the peloric variant is often presented as an example of an epiallele that has persisted for several hundreds of generations, there is actually no indication that this is the case. In fact, peloric plants have low fertility and the molecularly characterized peloric variant is therefore unlikely to descend from the original specimen described by Linnaeus. Nonetheless, the eighteenth-century French botanist Adanson noted that Linnaeus's peloric plants were unstable (15). This last observation suggests that Linnaeus's specimen was also caused by an epimutation, which in turn would indicate that the *Lcyc* locus or a specific allele at this locus is particularly prone to epiallelic variation. Thus, characterizing the precise genomic structure of different alleles of the *Lcyc* locus may prove useful in identifying the molecular determinants of frequent epimutability.

Another example of a spontaneous epimutation causing a striking phenotypic alteration is *Cnr* in tomato. *Cnr* mutant plants produce colorless, non-ripening fruits, and genetic analysis indicates that the mutant phenotype is caused by a recessive mutant allele at the *LeSPL-CNR* locus (83). However, as with the peloric variant of the toadflax, the *Cnr* phenotype could not be associated consistently with DNA sequence variation in or around *LeSPL-CNR*. Instead, the mutant phenotype was systematically associated with extensive DNA methylation of the promoter region and reduced expression of *LeSPL-CNR* (83). Moreover, methylome analysis of the *Cnr* mutant and a normal cultivar indicates that the region immediately upstream of the promoter of *LeSPL-CNR* corresponds to a TE that is heavily methylated in both strains (83, 161). As it has been shown that DNA methylation often spreads from TEs into adjacent sequences in *Arabidopsis* and maize (1, 33), it is very likely that the *Cnr* mutant epiallele was generated through a similar process (**Figure 1*a***).

In maize, epimutable alleles were first described by Barbara McClintock (90), who studied the effect of the *Suppressor-mutator* (*Spm*) transposon on gene expression, and Alexander Brink (16), who coined the term paramutation to describe allelic interactions leading to non-Mendelian inheritance patterns. Extensive molecular studies of paramutation at several loci have shown that

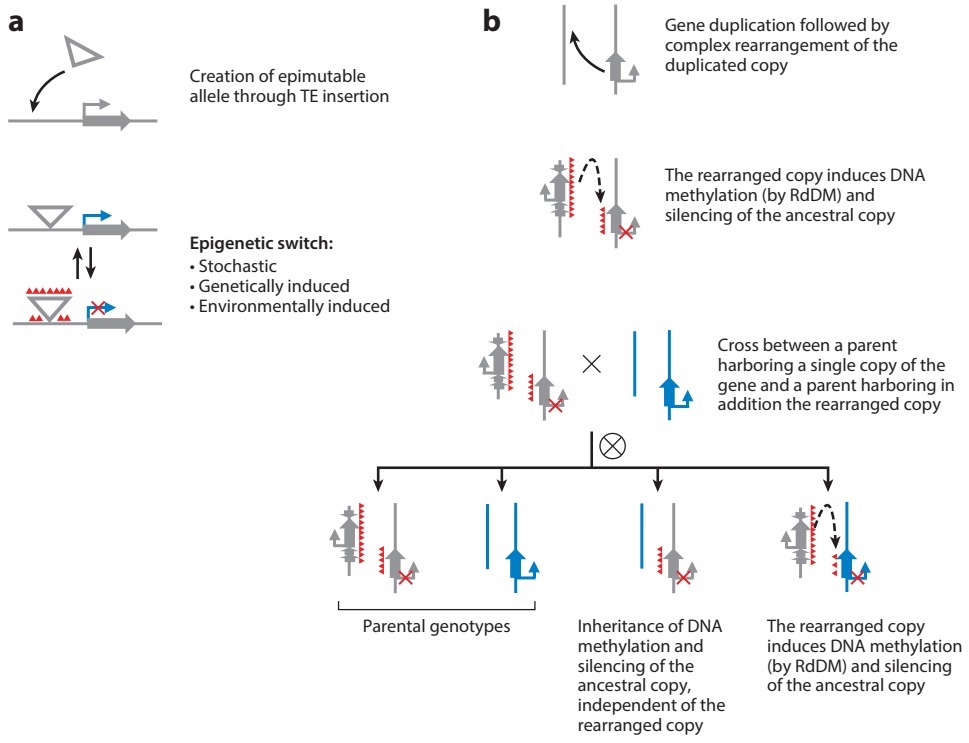

Figure 1

Transposable element (TE)- and repeat-associated epimutable alleles. (*a*) Creation of an epimutable epiallele by the insertion of a TE (*gray triangle*) near an active gene (*thick gray arrow*). The TE is initially in the unmethylated form, and the insertion may alter the expression (*blue arrow*) of the nearby gene. After an indeterminate number of generations, the TE becomes methylated and DNA methylation may also spread into adjacent sequences. DNA methylation (of the TE and/or downstream sequence) alters the expression of the gene (*red X over blue arrow*), thus creating a heritable epiallelic variant, which may switch back and forth stochastically or through the influence of a genetic modifier or in response to environmental changes. (*b*) Creation of an epimutable epiallele through gene duplication and rearrangement of the duplicated copy. The rearrangement is associated with the production of siRNAs, which have the ability to guide DNA methylation in *trans* and silence the original gene copy. The F2 population is derived from the F1 progeny of a cross between a plant with the gene duplication and a plant without it. DNA methylation and silencing of the original copy are stably inherited even in the absence of the rearranged copy, which can again methylate in *trans* and silence an unmethylated gene copy. Abbreviations: RdDM, RNA-directed DNA methylation; siRNA, small interfering RNA; TE, transposable element.

heritable differences of DNA methylation over repeat sequences are involved and that the fully methylated epialleles impose their methylation on the less methylated, often unstable epialleles when the two types of epialleles are brought together through a cross (2). As for *Spm*, we now know that the change of phase responsible for epimutability is associated with heritable changes in DNA methylation that occur either spontaneously or following the repeated confrontation of a so-called cryptic, fully methylated *Spm* copy with an active, partially unmethylated element (37). Another maize TE, called *Mutator*, also often creates epimutable alleles, and here again, heritable changes in DNA methylation are involved (85).

Three natural, epimutable, or potentially epimutable alleles have been reported that have agronomically important phenotypic consequences, and all three involve differences of DNA

methylation mediated by TE or other repeat sequences. In the case of the *Epi-dwarf* allele of rice causing dwarfism, there is clear evidence that it is metastable and that it can in fact be inherited as distinct epialleles (96). In contrast, the demonstration remains to be established that some alleles at the *g* and *VTE3*(*1*) loci, which condition, respectively, the production of unisexual flowers in melon (87) and vitamin E content in tomato (107), are subject to heritable epiallelic variation in nature.

Finally, several epimutable alleles have been described in *Arabidopsis*, and they all involve TEs or other repeat sequences (146). *FWA* is the best characterized, with a tandem repeat that is partially derived from an ancestral TE insertion forming part of the gene promoter. DNA methylation of the tandem repeat is responsible for the silencing of *FWA* during most of the plant life cycle. In the endosperm, however, the repeats are unmethylated and the gene is active on the maternally derived chromosome. Stably inherited hypomethylated *FWA* alleles can be recovered following experimental induction of loss of DNA methylation and these are associated with much delayed onset of flowering (38), but naturally hypomethylated epialleles have not been documented (38, 120, 140). In contrast, *PAI* and *FOLT1* genes vary naturally in their DNA methylation and expression status, with phenotypic consequences (6, 31). However, epiallelic variation at *PAI* and *FOLT1* results from sequence rearrangements elsewhere in the genome (**Figure 1*b***) and given that *Arabidopsis* is mostly self-fertilizing, epiallelic variation at these two loci does not segregate independently of the inducers in nature. In fact, the only known case of possible natural epiallelic variation in *Arabidopsis* is at *QQS*, which encodes a small protein involved in starch metabolism. Intriguingly, *QQS* is a recent de novo–formed gene located in a TE-rich region, which suggests that genes arising through spurious transcription may be particularly prone to epigenetic variation until they evolve proper regulatory sequences (125).

DNA METHYLATION LANDSCAPES

With the advent of whole-genome sequencing following bisulfite treatment of DNA, which converts unmethylated cytosine to uracil, it is now possible to determine the methylation status of every single cytosine within genomes. In fact, the first two methylome studies in eukaryotes were performed in *Arabidopsis* (21, 79). These two studies together with previous work performed using DNA tiling arrays revealed that the genomic distribution of DNA methylation is shaped largely by TE sequences in *Arabidopsis* (21, 78, 79, 158, 162). Methylome studies carried out in other plants have since indicated the generality of this conclusion. Thus, although the compact *Arabidopsis* genome (approximately 125 Mb) and other relatively small plant genomes are characterized by a higher density of TE sequences and DNA methylation in pericentromic regions (19, 21, 77, 79, 99, 123, 161), this is not the case for much larger plant genomes, such as of maize (approximately 2.3 Gb), which instead show a high density of TE sequences and DNA methylation throughout the chromosome arms (42, 99, 109). Why TE sequences accumulate between genes in only some species remains enigmatic, especially because DNA methylation over TEs is known to spread to adjacent genes and affect their expression in both *Arabidopsis* and maize (1, 33, 51).

In all vascular plants analyzed to date, TE sequences tend to exhibit methylation over their entire length and in the three possible nucleotide contexts, CG, CHG, and CHH (where H = C, A, or T). However, levels of methylation are almost always much higher at CG than CHG and especially CHH sites (typically >80%, 20–60%, and <20%, respectively) (74, 99). Furthermore, DNA methylation of TE and other repeat sequences is usually associated with dimethylation of lysine 9 of histone H3 (H3K9me2), a heterochromatic mark that is highly conserved among eukaryotes, as well as with the presence in variable abundance of matching small interfering RNAs (siRNAs). Together, these three features contribute to the stable silencing of TEs, which are in effect portable units of heterochromatin (78).

Although TE sequences are the major determinants of the DNA methylation landscape in plants, genes also contribute appreciably through so-called gene body methylation (gbM). Indeed, genes with moderate expression levels tend to be methylated at CG sites (as well as CHG sites in conifers) over part of their transcribed region (66, 99, 132, 133, 156). Furthermore, methylated genes tend to be the same among vascular plants, suggesting functional significance (132, 133). GbM could serve to stabilize transcription by preventing deposition of the histone variant H2A.Z, maintaining nucleosome phasing, suppressing intragenic cryptic promoters, or regulating alternative splicing (22). However, in the absence of strong evidence supporting any of these possibilities, it is equally plausible that gene body methylation is merely a by-product of transcriptional activity over specific genes, with no functional consequences (135). Moreover, the flowering plants *Eutrema salsugineum* and *Conringia planisiliqua* lack gbM altogether, which is also consistent with it being inconsequential (9).

DNA METHYLATION AND DEMETHYLATION MECHANISMS

Classically, DNA methylation mechanisms are divided into those responsible for establishing this modification and those involved in maintaining it. DNA methylation can also be lost through active or passive mechanisms, and it is the interplay between methylation and demethylation that defines the methylome of a given cell.

Establishment

Little is known about the way genes acquire gbM (9). In contrast, and although many questions remain, we now have a reasonable understanding of how DNA methylation is established over TE and other repeat sequences. In particular, plants have evolved a unique de novo DNA methylation pathway called RNA-directed DNA methylation (RdDM) that specifically targets these. Factors involved in RdDM have been identified mainly through extensive genetic analyses in *Arabidopsis*, and the picture that emerges is one of multiple, often interconnected steps (74, 89). In its current form, the model for canonical RdDM entails the initial production of short (<45 nt) primary transcripts that are generated by a plant-specific RNA Pol II derivative, Pol IV (11, 157), or possibly Pol II in some cases (152). These short transcripts are then converted to double-stranded RNAs (dsRNAs) by the activity of the RNA-dependent RNA polymerase RDR2. These short dsRNAs are diced by the Dicer-like protein DCL3 from either end to generate a single 24-nt–long siRNA in each case, which is loaded into the Argonaute protein AGO4 or its close homologs AGO6 and AGO9, depending on targets or cell types (11, 157). A Dicer-independent route also exists (151), which involves the loading of the double-stranded precursor RNAs into AGO4 and the subsequent trimming of their exposed 3′ end by a 3′-to-5′ distributive exonuclease, also ultimately generating 24-nt siRNAs (152). These 24-nt siRNAs are called sidRNAs to distinguish them from the Dicer-dependent 24-nt siRNAs (152), but both types serve to guide AGO4 to matching DNA targets. Guiding is thought to occur through the formation of an RNA-RNA intermediate with so-called scaffold transcripts generated by a second plant-specific RNA Pol II derivative, Pol V. However, a direct RNA-DNA interaction is not ruled out. Either way, guiding also brings AGO4 into physical contact with Pol V (35, 75), and genetic as well as biochemical evidence suggests that it is at this stage that DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), the main *Arabidopsis* homolog of the mammalian de novo DNA methyltransferase (DMTase) Dnmt3, is recruited to its target loci (163). The underlying mechanism is unclear but involves the bridging of AGO4 and DRM2 by the protein RDM1 (39). Unlike Dnmt3, DRM2 methylates Cs in all sequence contexts, not just at CG sites (3, 74, 89).

Several observations indicate that Pol IV and Pol V recruitment depends on previous DNA methylation, which therefore calls into question the function of canonical RdDM in de novo rather than maintenance DNA methylation (62, 73). Indeed, an alternative RdDM pathway has been uncovered that may fulfill the de novo function, as it starts with transcripts that are thought to be produced by Pol II. Furthermore, this alternative pathway involves RDR6 as well as DCL2 and DCL4 to give rise to 21- to 22-nt rather than 24-nt siRNAs (91, 92, 100, 106). This pathway, called RDR6-RdDM, is therefore reminiscent of the post-transcriptional gene silencing pathway except that here the 21- to 22-nt siRNAs associate with AGO4 or AGO6 to direct DNA methylation rather than with AGO1 to direct mRNA degradation. As RDR6-RdDM does not require Pol IV, it has been proposed to act upstream of canonical RdDM, thus leading to the notion that de novo DNA methylation may occur in two steps, initiation and establishment (12, 92, 100). However, like canonical RdDM, RdR6-RdDM requires Pol V, which suggests either that Pol V can in fact be initially recruited in a DNA methylation-independent manner or that Pol II also produces scaffold transcripts (62, 92). Although there is evidence for the latter possibility (160), further experiments are required to rule out a role for Pol V in the initial step of RdDM (89). Finally, it is not clear how general this alternative pathway is in initiating DNA methylation, notably because a third RdDM pathway involving RDR6 and DCL3 was shown to be responsible for triggering DNA methylation and silencing of newly transposed copies of the retrotransposon *Evadé* (84).

Maintenance

Once established, DNA methylation is maintained through DNA replication in a process called DNA methylation maintenance. Unlike mammals, which methylate mainly CG sites and have only one maintenance DMTase, Dnmt1, plants possess several distinct DMTases that are responsible for maintaining methylation at either CG, CHG, or CHH sites (74). In *Arabidopsis*, symmetrical CG methylation is maintained over both genes and repeat sequences almost exclusively by MET1, which is the plant homolog of Dnmt1 and functions similarly to it by recognizing, with the help of the accessory protein VIM1, hemimethylated CGs created by DNA replication (74). In contrast, maintenance of CHG methylation relies on the chromomethylases CMT3 and to a lesser extent CMT2 (130, 131), which are plant-specific DMTases characterized by the presence of a chromodomain in the catalytic domain. Unlike at CGs, maintenance of methylation at CHGs does not rely on the recognition of hemimethylated sites but rather on a self-reinforcing loop between H3K9me2 and CHG methylation. Specifically, CMT2 and CMT3 bind H3K9me2 through their chromodomain, whereas the H3K9 methyltransferases KYP/SUVH4, SUVH5, and SUVH6 bind through their SRA domain to methylated CHGs (29). In the case of CHH methylation, template-based maintenance is excluded because of the asymmetry of the trinucleotide sequence. Instead, CHH methylation is maintained by canonical RdDM over short TEs as well as the extremities of long TEs and by CMT2 for the internal part of long TEs (131, 155). This scheme is not universal however, because CMT2 or CMT3 are absent in some angiosperms (9, 155) and because methylation of CG sites shows different degrees of symmetry between species (99, 149). Furthermore, although DNA methylation is very well maintained at the level of whole TEs or genes (5, 66, 119, 120), it is important to remember that individual Cs rarely reach 100% methylation, even at CG sites.

Maintenance of DNA methylation over TE and other repeat sequences also requires the action of other factors, such as the ATP-dependent chromatin remodelers DDM1 and DRD1. DDM1 is the homolog of mammalian Lsh (98) and likely facilitates access of DMTases, especially CMT2, to H1-containing heterochromatin (155). DDM1 may also be involved in DNA methylation-independent activities (56). As for DRD1, which has no mammalian homolog, it is involved in

canonical RdDM (65, 72) and is thus mainly responsible for maintaining CHH methylation over short TEs and the extremities of long TEs (155). The action of these two chromatin remodelers further highlights the intimate relationship between DNA methylation and other aspects of chromatin structure (74).

Demethylation

Without continuous maintenance or de novo activities, DNA methylation is progressively diluted through replication and ultimately lost in a process called passive demethylation. Removal of DNA methylation can also be achieved through active mechanisms, which differ markedly between plants and mammals. Indeed, whereas mammals rely on TET enzymes to demethylate DNA (68), plants have no TET homologs and use DNA glycosylases instead. *Arabidopsis* has four such enzymes, ROS1, DME, DML2, and DML3, which excise methylated cytosines (74). The genes encoding ROS1, DML2, and DML3 are expressed throughout development and are to a great extent functionally redundant, with their main function likely being to maintain genic regions containing TE or other repeat sequences free of DNA methylation (79, 105). In contrast, *DME* is expressed exclusively in the companion cells of the egg and pollen (i.e., the central cell and the vegetative cell, respectively). *DME* is essential for the extensive maternal- and more limited paternal-specific DNA demethylation of TEs that underlies genomic imprinting in the endosperm (17, 40, 53). Finally, it should be mentioned that *Arabidopsis* uses IBM1, a jumonji-domain protein with putative histone H3 lysine 9 demethylase activity, to prevent CHG methylation over genes (54, 117).

DNA METHYLATION DYNAMICS DURING SEXUAL REPRODUCTION

In comparison to mammals, which extensively reprogram their methylome in the male and female germlines as well as in the early embryo, starting with the zygote (122), plants appear to undergo much less DNA methylation reprogramming during their life cycle (67). Indeed, *Arabidopsis* shows substantial loss of overall DNA methylation only in the central cell and the endosperm. This demethylation is DME dependent, as mentioned above, and affects mainly CG sites of TEs (40). Likewise, some DME-dependent CG demethylation takes place on the paternal side, in the vegetative nucleus (VN) of pollen grains, but affects comparatively few TEs (17, 53). In addition to these two instances of reduced overall DNA methylation, which concern cells that do not contribute directly to the next generation, there is also an almost complete absence of CHH methylation in sperm cells (SCs) (17). Based on indirect evidence, the zygote and early embryo may exhibit a methylome similar to that of SCs (52, 67). Thus, although MET1 and CMT3 are barely detectable in the egg cell (63), it appears that neither CG nor CHG methylation is actually reprogrammed across generations to any significant extent. In other words, TEs are likely transmitted through the two parental germlines in their almost fully methylated state (minus CHH methylation), with CG and CHG methylation likely being maintained immediately upon fertilization, as both MET1 and CMT3 are readily detected from the zygote onward (63). In contrast, CHH methylation increases progressively as the embryo develops, presumably in an RdDM-dependent manner (52, 63). Indeed, this progressive reestablishment during embryogenesis could be initially aided by the 21-nt siRNAs present in the SCs, which are thought to originate from hypomethylated TEs in the VN (17, 127). The 24-nt siRNAs that are produced outside the embryo (97), either in the seed coat or the endosperm, may also contribute, although a direct demonstration of their transport to the embryo is lacking (52).

The absence of extensive reprogramming of CG and CHG methylation over TE sequences in the germ cells as well as in the early embryo is also supported by the fact that mutants deficient in RdDM have near-normal patterns of CG and CHG methylation (131). Nonetheless, RdDM plays

an essential role in the case of accidentally demethylated TEs, as it allows a fraction of them to regain normal DNA methylation (136). For reasons that are still unclear, remethylation is typically achieved gradually over several reproductive cycles rather than at once (135, 136), an observation that is reminiscent of the phenomenon of transposon cycling in maize (36).

All of the available evidence suggests, therefore, that the methylome of plants is reinforced rather than reset or reprogrammed during sexual reproduction, which would be consistent with the relatively minor role of DNA methylation in the regulation of genes in plants compared to mammals.

EXPERIMENTAL INDUCTION OF HERITABLE DNA METHYLATION VARIANTS GENOME-WIDE

The first two plant genes known to be involved in DNA methylation are *DDM1* and *MET1*, which were isolated in a forward genetic screen for *Arabidopsis* mutants with reduced DNA methylation of centromeric repeats (64, 144). The loss of DNA methylation in both *ddm1* and *met1* (initially called *ddm2*) affected other sequences as well and, remarkably, it persisted after segregation of the mutant alleles (64, 144). This suggested for the first time the lack of extensive DNA methylation reprogramming in plants.

To determine the extent to which the loss of DNA methylation induced by *ddm1* or *met1* can be inherited, two populations of epigenetic recombinant inbred lines (epiRILs) were established, starting with a cross between mutant and wild-type parents of the same genetic background. F2 progeny homozygous for the wild-type allele were selected and used to generate multiple independent lines through six rounds of propagation by single seed descent (61, 110) (**Figure 2*a***). Methylome analysis was conducted for three *met1*-derived epiRILs at the F8 generation, and recombined parental chromosomal segments were readily identified in each case. However, non-parental DNA methylation differences were also detected in very high numbers across the genome (110), presumably because of the misregulation in *met1* mutants and in subsequent generations of at least two genes, *ROS1* and *IBM1*, involved directly or indirectly in DNA demethylation (88, 113, 114). Moreover, although TEs are not mobilized in *met1* despite their massive transcriptional reactivation, a handful of TEs do transpose in the *met1*-derived epiRILs (94, 110), in part because of further DNA methylation changes that take place in the F1 (113).

In contrast to the *met1*-derived epiRILs, the *ddm1*-derived epiRILs exhibit relatively few non-parental DNA methylation differences (55), consistent with the absence in *ddm1* of any appreciable misregulation of *ROS1* or other genes involved in DNA methylation control (131, 136, 155). At the same time, because TE sequences lose methylation at CHG and CHH sites as well as CG sites in *ddm1* (136, 155), more TEs are mobilized in *ddm1* and the *ddm1*-derived epiRILs than in *met1* and the corresponding epiRILs (24, 46, 61, 84, 95, 126, 138). Importantly, a methylome analysis was performed on 123 *ddm1*-derived epiRILs (generation F8), thus enabling a systematic assessment of the transgenerational stability of *ddm1*-induced loss of DNA methylation (23). Over 2,600 regions of differential DNA methylation between the *ddm1* and wild-type parental lines were examined, which as expected align almost exclusively with TE sequences. Approximately two-thirds of these differentially methylated regions (DMRs) are present mainly or only in the methylated state in the epiRILs (generation F8), indicating partially or fully penetrant reversion to wild-type DNA methylation. Furthermore, these parental DMRs are characterized by an abundance of matching 24-nt siRNAs (23), which is consistent with RdDM being responsible for the efficient reacquisition of DNA methylation over these specific regions (136). In contrast, the remaining parental DMRs segregate according to Mendel's laws, indicating stable inheritance of the *ddm1*-induced hypomethylated state over hundreds of regions across the genome even after eight generations. Indeed, a genetic map could be constructed based on these stable parental DMRs, which turned

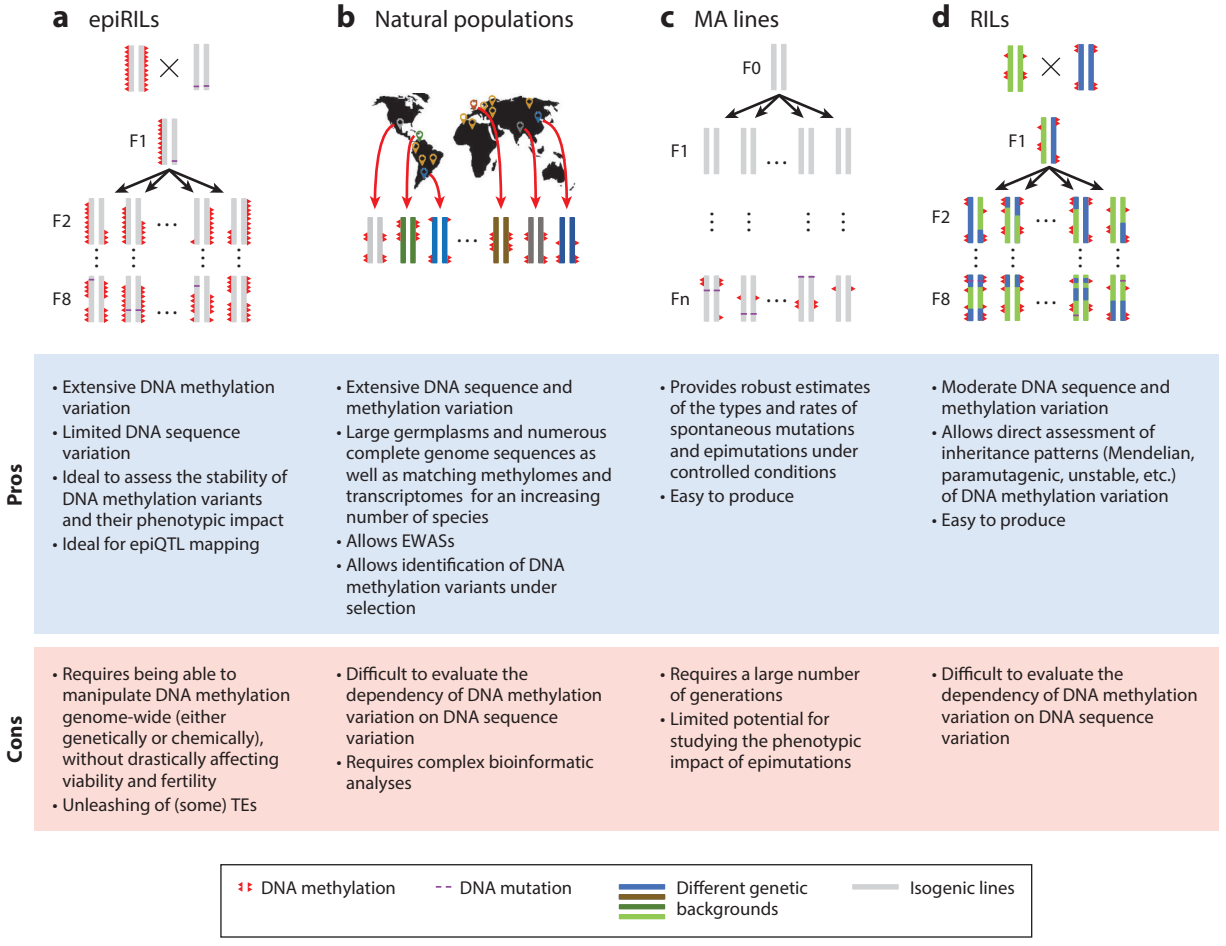

Figure 2

Experimental versus natural populations for transgenerational epigenetic studies: pros and cons. (*a*) A plant with a mutation (such as *ddm1* or *met1*) that induces altered levels of DNA methylation genome-wide is crossed with an isogenic wild-type plant. A single F1 plant is selfed or backcrossed to the wild-type parent to segregate out the inducing mutation, and nonmutant F2 progeny are used to obtain a population of isogenic epigenetic recombinant inbred lines (epiRILs) by repeated selfing (six generations) and single seed descent. (*b*) Strains are collected from the wild and grown under controlled conditions. (*c*) Several independent lines are propagated for a large number of generations by repeated selfing and single seed descent from a unique parent to generate so-called mutation accumulation (MA) lines. (*d*) Two different strains are crossed, and the F2 progeny is used to obtain a population of RILs by repeated selfing (six generations) and single seed descent. Note the accumulation of mutations that also occurs during the propagation of RILs and epiRILs, especially as a result of transposable element (TE) mobilization in the latter case. Abbreviations: EWASs, epigenome-wide association studies; epiQTL, epigenetic quantitative trait locus.

out to be very similar to classical genetic maps built using DNA sequence variants between natural accessions (23, 59).

GENOME-WIDE ASSESSMENT OF NATURAL DNA METHYLATION VARIATION

An increasing number of studies have explored DNA methylation variation in nature (**Figure 2*b***). The first extensive survey was performed in *Arabidopsis* using a methyl-sensitive enzyme and

Southern blot analysis to determine the DNA methylation level across accessions of the large tandem arrays of rRNA genes that form the nucleolus organizer regions (NORs). Natural variation in NOR methylation was readily observed and found to be heritable (112, 153). Through a quantitative genetics approach, both *cis*- and *trans*-acting factors associated with this variation were identified, with the NORs themselves being the most significant determinants (112, 150). As the number of rRNA genes correlates strongly with DNA methylation levels, this is likely the main causal factor, although heritable DNA methylation variation per se may also be involved (150).

The first chromosome-wide analysis of natural DNA methylation variation in *Arabidopsis* was based on DNA microarray technology and thus did not enable CG, CHG, and CHH methylation to be distinguished. Nonetheless, this analysis revealed that DNA methylation over genes varies greatly between accessions, whereas it is much more stable over TE sequences (140). Whole-genome analyses at the resolution of single cytosines have since been performed for hundreds of natural accessions, confirming this picture and in addition providing detailed qualitative as well as quantitative assessments of natural variation in DNA methylation (66, 120).

In the two most comprehensive studies to date, 140 and an additional 975 accessions taken from across the globe were investigated (66, 120). For several accessions, the methylome of mixed-stage inflorescences was compared to that of leaves, and few DNA methylation differences were detected, thus confirming that DNA methylation patterns are largely invariant between tissues or organs. In contrast, extensive variation was observed among accessions, but mostly at the single cytosine level (**Table 1**). Specifically, any two accessions differ from each other by between 90,000 and 500,000 differentially methylated positions [DMPs; also called single methylated polymorphisms (SMPs)], with CHH-DMPs being the most abundant (64% of all DMPs versus 23% and 16% for CG- and CHG-DMPs, respectively) (120). These values are similar to those reported for single nucleotide polymorphisms (SNPs) among accessions (18, 80) and phylogenies based on DMPs or SNPs are highly correlated (120), which suggests that DMPs are to a large extent caused by SNPs in *cis* or in *trans*. However, although SNPs are enriched over TE and other repeat sequences and therefore tend to accumulate in pericentromeric regions (18, 80), the patterns of DMP diversity are much more complex (120). CG- and CHG-DMPs are less abundant and more often methylated in pericentromeric than in gene-rich regions, which reflects the robustness of CG and CHG methylation over TEs. In contrast, CHH-DMPs are most abundant over TEs and tend to be present in the unmethylated form, consistent with CHH methylation being almost exclusively found at these sequences and at low levels (<20%) over individual sites.

Unlike DMPs, DMRs are relatively few between any two accessions, as only approximately 53,000 DMRs were identified among 140 accessions (120). However, in the majority of cases, DMPs and DMRs do not coincide. This last observation together with the fact that differential methylation of individual cytosines is not known to have any effect in vivo suggests that most natural DMPs are functionally inconsequential. Furthermore, most DMRs are located within genes, in agreement with previous observations (140), and are in the CG context only (CG-DMRs), as expected for gbM (66, 120). Although the higher prevalence of CG-DMRs within genes conflicts with the reported evolutionary conservation of gbM, it can be explained in part by local variations rather than by a presence/absence of methylation along genes. In any case, given the absence of evidence for a role of gbM (9), it is likely that most natural CG-DMRs in genes are functionally inconsequential as well.

The remaining DMRs, called C-DMRs, correspond mainly to TE sequences and are present mostly in the methylated state across accessions (66, 120), in keeping with previous observations (140). Like CG-DMRs over genes, C-DMRs over TEs tend to reflect variation in the degree of methylation rather than presence/absence of DNA methylation, although the possible confounding effect of variation in TE copy number between accessions should be considered. In addition,

Table 1 Epimutation rates in nature

Organism	Experiment	Locus or context	Epimutation rate (units)	Method	Reference(s)
Arabidopsis	Mutation accumulation lines	CG-DMP	1,000 (DMP/generation)	Bisulfite	5, 119
		Non-CG-DMP	<0.033 (DMP/generation)[a]	Bisulfite	5, 119
		CG-DMP (forward)[b]	2.56×10^{-4} [DMP/(CG site · generation)	Bisulfite	139
		CG-DMP (reverse)[c]	6.30×10^{-4} [DMP/(CG site · generation)]	Bisulfite	139
		CG-DMR	0.8–1.3 (DMR/generation)	Bisulfite	5, 119
		C-DMR	0.34 (DMR/generation)	Bisulfite	5, 119
	Accessions from North America	CG-DMP	717 (DMP/generation)	Bisulfite	47
		Non-CG-DMP	22 (DMP/generation)[a]	Bisulfite	47
		CG-DMR	2.6 (DMR/generation)	Bisulfite	47
		C-DMR	1.1 (DMR/generation)	Bisulfite	47
	Accessions collected worldwide	CG-DMP	0.17 (DMP/SNP)	Bisulfite	120
		Non-CG-DMP	0.60 (DMP/SNP)[a]	Bisulfite	120
Maize	RILs (F7 population)	DMR	4.6×10^{-6}–1.7×10^{-3} (DMR/generation)	meDIP-chip	76
Rice	F1 hybrids	DMP	8×10^{-3} [DMP/(cytosine · generation)][d]	Bisulfite	19
		DMR	75–340 (DMR/generation)[d]	Bisulfite	19
	Instability of *epi-dwarf* phenotype	*DWARF1* (forward)	0.13 (phenotypic switch/generation)	Phenotypic	95
		DWARF1 (revert)	0.27 (phenotypic switch/generation)	Phenotypic	95
Tomato	Instability of *Cnr* phenotype	*Cnr*	$<3.3 \times 10^{-4}$ (revertant plants/generation)	Phenotypic	83
Soybean	RILs (F3 population)	CG-DMR	127 (DMR/generation)[d]	Bisulfite	83
		C-DMR	41 (DMR/generation)[d]	Bisulfite	118

[a]Rates may be underestimated because of reduced statistical power to detect differences in DNA methylation at CHH and CHG sites.
[b]Rate of DNA methylation gain.
[c]Rate of DNA methylation loss.
[d]Rate includes stochastic epimutation as well as paramutation.
Abbreviations: DMP, differentially methylated position; DMR, differentially methylated region; meDIP-chip, immunoprecipitation of methylated DNA followed by microarray hybridization; RILs, recombinant inbred lines; SNP, single nucleotide polymorphism.

almost 9,000 genes overlap with C-DMRs in at least one of 846 accessions analyzed. However, although gbM is consistent across most of these accessions, this is not the case for TE-like DNA methylation within genes, which is typically a very rare occurrence for any given gene (66). Examination of this type of variant indicates that they most likely result from the presence of methylated, low frequency TE insertions within or close to genes and from the spreading of DNA methylation from these TE insertions into flanking sequences (108, 120).

Genome-wide association studies (GWASs) have revealed that CG-DMRs are usually not linked to DNA sequence variation and must therefore represent either inherent stochastic fluctuations in gbM or true heritable DNA methylation variants. In contrast, one-third of C-DMRs

are associated with local and distal sequence variation, indicating in these cases a possible causal relationship, such as the presence of a TE insertion, as mentioned above (1, 108, 120). For the remaining two-thirds of C-DMRs, the absence of association with DNA sequence variation could reflect bona fide heritable DNA methylation variants. Consistent with this possibility, a sizeable fraction (30%) of the stable DMRs identified in the *ddm1*-derived epiRILs, which by design are inherited independently of DNA sequence changes, overlap with natural C-DMRs (24).

DNA methylation variation among local *Arabidopsis* populations was also investigated. One study analyzed 150 accessions from Sweden and another 13 accessions from North America and the results were consistent with those obtained from the two worldwide surveys (30, 47). In addition, a *trans* modifier of DNA methylation specifically affecting CHH methylation was identified among the Swedish accessions and maps to the *CMT2* locus (30); however, no causal sequence variants were found. As the 13 American accessions are near clonal and have only recently diverged from each other, they could be used to assess methylome stability in natural settings in the absence of large-scale DNA sequence variation. Approximately 3,200 DMRs with extreme methylation differences were detected. These DMRs map predominantly in intergenic regions but are otherwise equally located in genes and TEs. Furthermore, these DMRs tend to be stably inherited and are often associated with DNA sequence variants (47). Thus, the methylome appears to be remarkably stable over short timescales in nature, and DNA methylation variation is likely caused in large part by DNA sequence variation.

To obtain direct estimates of the rate of spontaneous DNA methylation variation, methylome analyses were carried out on several *Arabidopsis* mutation accumulation (MA) lines (5, 119, 139) (**Figure 2*c***). DMPs accumulate in these lines at a rate of approximately 1,000 per generation, which is three orders of magnitude higher than the rate of DNA mutations (102). As in natural populations, most DMPs occur at CG sites within genes and one-third of these DMPs are shared among lines, implying that some CG-DMPs are recursive. These results indicate that DNA sequence variation is unlikely to be the cause of epimutability at individual cytosines, which in turn suggests that the strong correlation observed between DMPs and SNPs in natural accessions (120) simply reflects common ancestry between the two types of variation. In contrast to DMPs, DMRs are as rare as DNA sequence mutations in the MA lines. Moreover, DMRs are mostly CG-DMRs and mainly affect genes (5, 119), suggesting that the vast majority of these spontaneous DMRs are functionally inconsequential.

In addition to these extensive analyses in *Arabidopsis*, more limited methylome surveys have been performed in maize, soybean, and rice, and all have revealed a similar picture (19, 32, 34, 76, 77, 118). In the case of maize and soybean, RILs were used (**Figure 2*d***), which confirmed that natural DMRs tend to cosegregate with DNA sequence variants. However, inheritance independent of such variants was proven for a few DMRs (32, 34, 76, 118).

LINKING HERITABLE DNA METHYLATION VARIATION TO PHENOTYPIC VARIATION GENOME-WIDE

As DNA methylation variants are typically associated with sequence polymorphisms in natural populations, assessing the heritability and impact on phenotypic variation of the former independently of the latter poses many technical difficulties (60). By design, the *met1*- and *ddm1*-derived epiRILs circumvent the issue of DNA sequence variation almost completely. Moreover, both *met1* and *ddm1* mutations induce a number of severe phenotypic alterations that are inherited independently of the inducing mutation, and some of these are caused by heritable DNA methylation defects (86). Thus, the *met1*- and *ddm1*-derived epiRILs provide a powerful system to investigate genome-wide the phenotypic effects of heritable DNA methylation variation

per se, using quantitative genetics. Although this avenue was not pursued with the *met1*-derived epiRILs, presumably because of the low number of lines obtained and the extensive instability of the methylome across generations (110), it proved extremely effective when applied to the large population of *ddm1*-derived epiRILs (24, 61, 69, 71, 116, 159). Indeed, a number of ecological, molecular, and physiological traits have been evaluated in this population of over 500 lines and most traits were found to vary heritably. Phenotypic variation among the *ddm1*-derived epiRILs is usually of smaller amplitude than that found in classical RILs (24, 61, 69, 71, 116, 159). Nonetheless, heritability in the *ddm1*-derived epiRIL population reflects that observed among natural accessions, thus suggesting that epiallelic variation contributes to heritable differences in complex traits in nature (116). Moreover, using the genetic map of the *ddm1*-derived epiRILs and linkage analysis, several quantitative trait locus (QTL) intervals defined by stable DMRs were identified for a variety of traits. As TEs are mobilized in both *ddm1* and the epiRILs, whole-genome sequencing was used to rule out the possibility that new TE insertions are the cause of these trait variants (24, 69). As in classical QTL mapping experiments, the next challenge is to identify the causal variants, which may be greatly aided by the development of DNA methylation editing methods based on the clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 system (137, 143).

Based on the results obtained with the *ddm1*-derived epiRIL population, it can be safely argued that at least in plants, DNA methylation variants underlie part of the natural variation in complex traits. Moreover, because by definition DNA methylation and sequence variants need not align with each other, the former likely explain some of the so-called missing heritability that often plagues GWASs (14, 147). Although in their infancy, epigenome-wide association studies (EWASs) should therefore prove extremely useful in identifying causal DNA methylation variation. In fact, an EWAS has already been applied successfully in one case, to identify the epigenetic alteration that underlies the metastable mantled somaclonal variant of oil palm (101). Genome-wide DNA methylation profiling of several dozen somaclonal ramets with either a mantled or a normal phenotype was carried out and a single DMR, which in this case spans a TE, was consistently associated with the phenotypic variation. Further investigation demonstrated that hypomethylation of this TE, a retroelement called *Karma*, leads to the expression of an alternative spliced form of the *DEFICIENS*-like homeotic gene *MANTLED*, which in turn triggers homeotic transformation, parthenocarpy, and loss of oil yield (101). In addition, two EWASs have been carried out together with a GWAS in *Arabidopsis* to identify the contribution of DNA methylation variation to differences in gene expression among natural accessions (66, 93). Results of these studies indicate that most DNA methylation variants with significant association are located close to the gene in question (i.e., in *cis*) and are in linkage disequilibrium with SNPs, thus preventing a rigorous assessment of the respective contribution of DMRs and SNPs to gene expression differences. Nonetheless, several of the identified *cis*-DMRs are uncorrelated with SNPs, indicating that at least in these cases they are bona fide epigenetic variants, with potential effects on gene expression (93).

GENOME-WIDE ASSESSMENT OF THE ROLE OF THE ENVIRONMENT IN HERITABLE DNA METHYLATION VARIATION

For a variety of reasons, transgenerational epigenetic variation is often considered as a possible response of organisms to environmental changes (4, 25, 49, 13, 111). Hence, numerous studies have been conducted to determine the role of the environment in inducing changes in DNA methylation that are heritable, with mixed results.

For instance, *Arabidopsis* plants propagated under harsh conditions (high salt) showed a slightly higher rate of heritable methylation variation at CG sites (CG-DMPs as well as CG-DMRs), but the increase in the rate of CG-DMRs was of the same order as that of DNA mutations (58). In

a more comprehensive study, it has been shown that hyperosmotic stress repeated over several generations induces robust hypermethylation of CHG and CHH sites over TEs, which affects the expression of genes associated with adaptive phenotypic stress responses (148). However, these DNA methylation variants and stress responses are only transmitted to the next generation and specifically through the female germline because of extensive DME-dependent erasure of non-CG methylation in the male germline (148).

In contrast, *Arabidopsis* plants treated with either virulent or avirulent bacteria, the bacterial flagellin-derived peptide flg22, or salicylic acid display widespread hypomethylation of TEs (28, 154). Furthermore, this hypomethylation, which is likely caused by a reduction in RdDM activity (154), is associated with increased expression of several immune response genes, indicating that it is functionally important (28, 154). Although inheritance of these changes has not been investigated, it is suggested by observations indicating that the immune response to pathogen attack can be transmitted over at least two generations and that this response is mimicked by mutants deficient in non-CG methylation (81, 82).

Finally, it has been shown that in rice, but not in *Arabidopsis*, the upregulation of genes caused by phosphorus deprivation leads to the hypermethylation of adjacent TEs by an unknown mechanism that does not involve RdDM components (121). However, this hypermethylation is not transmitted to the next generation; instead, it is reversed as the upregulated genes return to their initial, low-expression state once phosphorus deprivation has ceased (121).

In addition to these experimental studies, two population-wide surveys have been performed with the explicit aim of exploring the extent to which natural environments may shape the methylome. Using near-clonal North American *Arabidopsis* accessions, which diverged less than a few hundred years ago, the accumulation of DNA methylation variants under natural conditions was assessed. Although these accessions were sampled from a wide range of environments, the rate of DNA methylation variants was not higher than that measured using MA lines propagated in a greenhouse (47). In contrast, methylome analysis of 150 Swedish accessions grown at two different temperatures (10°C and 16°C) revealed increased CHH methylation over TE sequences at 16°C, but only in some accessions. GWASs identified a significant association with allelic variation at the *CMT2* locus (30), which in turn correlates with the environmental conditions where the accessions grow naturally (124). These results therefore suggest that CHH methylation variation, which by itself cannot be inherited (see above), is a plastic response to environmental changes and is under genetic control. Whether or not this plastic response is adaptive remains to be determined.

Plant species can be propagated artificially through tissue culture, which represents an extreme form of environmental challenge and leads to so-called somaclonal variation. Methylome studies in several species have revealed that tissue culture induces a massive loss of DNA methylation and associated siRNAs over TEs (101, 128, 129, 134, 142), which in the case of oil palm could be demonstrated to underlie the mantled somaclonal variation, as mentioned above (101). Importantly, the mantled phenotype caused by hypomethylation of the *Karma* TE inserted in the *MANTLED* gene reverts at high frequency and revertants show normal *Karma* DNA methylation (101), presumably because *Karma* is efficiently targeted by the DNA methylation reinforcement activities that are associated with sexual reproduction. Some plant species, such as dandelions, can produce seeds asexually in a process termed apomixis. Whether DNA methylation dynamics across generations differ between the apomictic and sexual modes of reproduction is not known. Nonetheless, experiments with apomictic dandelions grown in different environments have shown that environmentally induced DNA methylation variation can be transmitted to the next generation, with potential phenotypic consequences (141). However, maternal effects cannot be ruled out as subsequent generations were not analyzed.

SOME FINAL CONSIDERATIONS

It is now clear that plants have a large potential for heritable DNA methylation variation over TE or other repeat sequences as well as adjacent regions and that this variation by itself may have phenotypic consequences. There is also convincing evidence that part of this potential is realized in nature, even though this cannot be demonstrated easily, because of the confounding effect of sequence variation. Furthermore, we are beginning to understand what determines the variable transgenerational stability of DNA methylation variation, which ranges from a few to tens and possibly hundreds or thousands of generations. However, we are far from knowing how much of this additional inheritance system actually contributes to heritable phenotypic variation in natural populations, nor do we know the role of the environment in the creation of environmentally induced epimutations, which seems less prevalent than was usually anticipated.

Despite these limitations, theoreticians have already integrated transgenerational epigenetic variation into population genetics models to determine conditions under which epimutable alleles, which are assumed to switch their state at high rates compared to mutations, could distort long-term evolutionary dynamics (27, 43–45, 57, 70, 103, 104). Here, we simply mention three models that illustrate different interesting properties of environmentally induced epimutable alleles in relation to the dynamics of adaptation and may in turn inform future empirical investigations of transgenerational epigenetic variation (**Figure 3**).

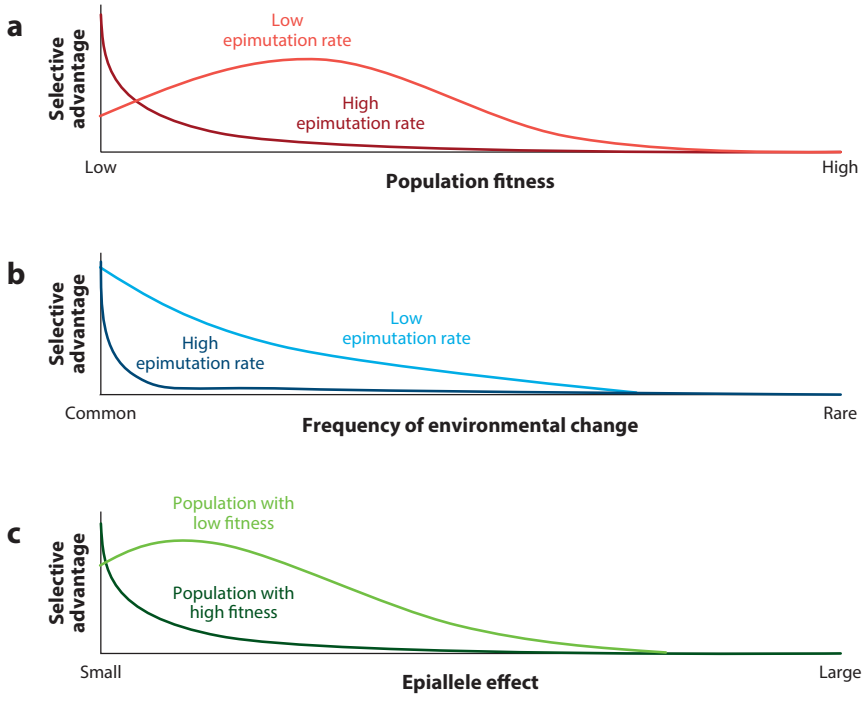

Figure 3

Selective advantage of transgenerational epigenetic variation. Selective advantage conferred by a novel epimutable allele (with high or low epimutation rate) in a hypothetical population as a function of (*a*) the population fitness or (*b*) the frequency of environmental changes. (*c*) Selective advantage conferred by a novel epimutable allele (that originated in a population with high or low fitness) as a function of the relative fitness effect of the epigenetic switch.

One model predicts that the phenotypic variation provided by the switch between epiallelic states is more advantageous when the population has a low or intermediate fitness value. A second model focuses on the effect of environmental cues on the selection of epimutatble alleles. It is shown that epimutability can be selected when the population faces contrasting environments on a regular basis, as this increases the frequency of matches between phenotypes and environment. Moreover, the more frequent or dramatic these regular environmental fluctuations are, the higher the number of distinct epimutable alleles that can be selected at a given locus (43–44) (**Figure 3*b***). In other words, the longer the fitness trajectory, the higher the selection coefficient for epimutable alleles is expected to be (103, 104) (**Figure 3*a***). A third model goes one step further by considering together epimutations and classical mutations. It predicts that the contribution of epimutations to adaptive walks (i.e., the paths across the fitness landscapes) is maximal when the alternative epialleles have minimal fitness effects, thus enabling the fine-tuning of phenotypes late in adaptive walks (70) (**Figure 3*c***), an interesting prediction given the low amplitude of the phenotypic variation observed experimentally with epiRILs (61). Nonetheless, this model also predicts that epimutations are more important early than late in adaptive walks.

Thanks to the power of genomics and epigenomics, combined with carefully designed experiments in natural as well as controlled environments, the contribution of transgenerational epigenetics to natural phenotypic variation will hopefully become better understood in the years to come.

SUMMARY POINTS

1. Plants may be particularly prone to transgenerational epigenetic variation, which entails heritable changes in DNA methylation.
2. Epiallelic variation is mostly associated with the presence of TE and other repeat sequences.
3. We propose that failed reinforcement rather than failed reprogramming of DNA methylation is responsible for the inheritance of DNA methylation variants and thus the creation of epimutations.
4. EpiRILs and natural accessions provide powerful complementary means for studying transgenerational epigenetics.
5. Direct evidence of the inheritance of environmentally induced changes in DNA methylation is still lacking, thus questioning the role of the environment in creating epiallelic variation.

FUTURE ISSUES

1. Our understanding of transgenerational epigenetics has been greatly aided by the creation of epiRILs in *Arabidopsis*. Additional epiRIL populations created using alternative inducers of heritable DNA methylation variation as well as multiple strains that differ from each other by a large number of TE insertion polymorphisms are needed in *Arabidopsis* and other species.

2. The difficulty of inducing heritable DNA methylation variation in a locus-specific manner has hampered studies of transgenerational epigenetic variation. Use of the CRISPR-Cas9 system for DNA methylation editing should remedy this and may also lead to important developments in plant breeding.
3. RdDM plays a major role in preventing transgenerational DNA methylation variation. However, we still have a very poor understanding of what determines the differential efficiency of RdDM over TEs and other repeat sequences.
4. Including natural DNA methylation variants as explanatory variables in GWASs may help resolve part of the problem of missing heritability.
5. TEs and other repeat sequences are typically overlooked in population genomics studies, which limits the exploration of epiallelic variation in nature. Efforts to include these sequences are therefore essential.
6. More work is required to determine the role of the environment in epiallelic variation.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Ahmed I, Sarazin A, Bowler C, Colot V, Quesneville H. 2011. Genome-wide evidence for local DNA methylation spreading from small RNA-targeted sequences in *Arabidopsis*. *Nucleic Acids Res.* 39(16):6919–31
2. Arteaga-Vazquez MA, Chandler VL. 2010. Paramutation in maize: RNA mediated trans-generational gene silencing. *Curr. Opin. Genet. Dev.* 20(2):156–63
3. Baubec T, Colombo DF, Wirbelauer C, Schmidt J, Burger L, et al. 2015. Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature* 520(7546):243–47
4. Baulcombe DC, Dean C. 2014. Epigenetic regulation in plant responses to the environment. *Cold Spring Harb. Perspect. Biol.* 6(9):a019471
5. Becker C, Hagmann J, Müller J, Koenig D, Stegle O, et al. 2011. Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature* 480(7376):245–49
6. Bender J. 2004. DNA methylation of the endogenous *PAI* genes in *Arabidopsis*. *Cold Spring Harb. Symp. Quant. Biol.* 69:145–53
7. Berry S, Dean C. 2015. Environmental perception and epigenetic memory: mechanistic insight through *FLC*. *Plant J.* 83:133–48
8. Bestor TH, Chandler VL, Feinberg AP. 1994. Epigenetic effects in eukaryotic gene expression. *Dev. Genet.* 15(6):458–62

9. Bewick AJ, Ji L, Niederhuth CE, Willing E-M, Hofmeister BT, et al. 2016. On the origin and evolutionary consequences of gene body DNA methylation. *PNAS* 113:9111–16
10. Bird A. 2007. Perceptions of epigenetics. *Nature* 447:396–98
11. Blevins T, Podicheti R, Mishra V, Marasco M, Wang J, et al. 2015. Identification of Pol IV and RDR2-dependent precursors of 24 nt siRNAs guiding de novo DNA methylation in *Arabidopsis*. *eLife* 4:1–22
12. Bond DM, Baulcombe DC. 2015. Epigenetic transitions leading to heritable, RNA-mediated *de novo* silencing in *Arabidopsis thaliana*. *PNAS* 112(3):201413053
13. Bossdorf O, Richards CL, Pigliucci M. 2008. Epigenetics for ecologists. *Ecol. Lett.* 11(2):106–15
14. Brachi B, Morris GP, Borevitz JO. 2011. Genome-wide association studies in plants: the missing heritability is in the field. *Genome Biol.* 12(10):232
15. Briggs D, Walters SM. 1984. *Plant Variation and Evolution*. Cambridge, UK: Cambridge Univ. Press. 2nd ed.
16. Brink RA. 1956. A genetic change associated with the *R* locus in maize which is directed and potentially reversible. *Genetics* 41(6):872
17. Calarco JP, Borges F, Donoghue MTA, Van Ex F, Jullien PE, et al. 2012. Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. *Cell* 151(1):194–205
18. Cao J, Schneeberger K, Ossowski S, Günther T, Bender S, et al. 2011. Whole-genome sequencing of multiple *Arabidopsis thaliana* populations. *Nat. Genet.* 43(10):956–63
19. Chodavarapu RK, Feng S, Ding B, Simon SA, Lopez D, et al. 2012. Transcriptome and methylome interactions in rice hybrids. *PNAS* 109(30):12040–45
20. Cleaton MAM, Edwards CA, Ferguson-Smith AC. 2014. Phenotypic outcomes of imprinted gene models in mice: elucidation of pre- and postnatal functions of imprinted genes. *Annu. Rev. Genom. Hum. Genet.* 15:93–126
21. Cokus SJ, Feng S, Zhang X, Chen Z, Merriman B, et al. 2008. Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature* 452:215–19
22. Coleman-Derr D, Zilberman D. 2012. Deposition of histone variant H2A.Z within gene bodies regulates responsive genes. *PLOS Genet.* 8(10):e1002988
23. Colomé-Tatché M, Cortijo S, Wardenaar R, Morgado L, Lahouze B, et al. 2012. Features of the *Arabidopsis* recombination landscape resulting from the combined loss of sequence variation and DNA methylation. *PNAS* 109(40):16240–45
24. Cortijo S, Wardenaar R, Colomé-Tatché M, Gilly A, Etcheverry M, et al. 2014. Mapping the epigenetic basis of complex traits. *Science* 343(6175):1145–48
25. Crisp PA, Ganguly D, Eichten SR, Borevitz JO, Pogson BJ. 2016. Reconsidering plant memory: intersections between stress recovery, RNA turnover, and epigenetics. *Sci. Adv.* 2(2):e1501340
26. Cubas P, Vincent C, Coen E. 1999. An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* 401(6749):157–61
27. Day T, Bonduriansky R. 2011. A unified approach to the evolutionary consequences of genetic and nongenetic inheritance. *Am. Nat.* 178(2):E18–36
28. Dowen RH, Pelizzola M, Schmitz RJ, Lister R, Dowen JM, et al. 2012. Widespread dynamic DNA methylation in response to biotic stress. *PNAS* 109(32):E2183–91
29. Du J, Johnson LM, Jacobsen SE, Patel DJ. 2015. DNA methylation pathways and their crosstalk with histone methylation. *Nat. Rev. Mol. Cell Biol.* 16(9):519–32
30. Dubin MJ, Zhang P, Meng D, Remigereau M-S, Osborne EJ, et al. 2015. DNA methylation in *Arabidopsis* has a genetic basis and shows evidence of local adaptation. *eLife* 4:e05255
31. Durand S, Bouché N, Perez Strand E, Loudet O, Camilleri C. 2012. Rapid establishment of genetic incompatibility through natural epigenetic variation. *Curr. Biol.* 22(4):326–31
32. Eichten SR, Briskine R, Song J, Li Q, Swanson-Wagner R, et al. 2013. Epigenetic and genetic influences on DNA methylation variation in maize populations. *Plant Cell* 25(8):2783–97
33. Eichten SR, Ellis NA, Makarevitch I, Yeh C, Gent JI, et al. 2012. Spreading of heterochromatin is limited to specific families of maize retrotransposons. *PLOS Genet.* 8(12):e1003127
34. Eichten SR, Swanson-Wagner RA, Schnable JC, Waters AJ, Hermanson PJ, et al. 2011. Heritable epigenetic variation among maize inbreds. *PLOS Genet.* 7(11):e1002372

35. El-shami M, Pontier D, Lahmy S, Braun L, Picart C, et al. 2007. Reiterated WG/GW motifs form functionally and evolutionarily conserved ARGONAUTE-binding platforms in RNAi-related components. *Genes Dev*. 21:2539–44
36. Fedoroff N. 1989. The heritable activation of cryptic suppressor-mutator elements by an active element. *Genetics* 121(3):591–608
37. Fedoroff N, Schläppi M, Raina R. 1995. Epigenetic regulation of the maize *Spm* transposon. *BioEssays* 17(4):291–97
38. Fujimoto R, Kinoshita Y, Kawabe A, Kinoshita T, Takashima K, et al. 2008. Evolution and control of imprinted *FWA* genes in the genus *Arabidopsis*. *PLOS Genet.* 4(4):e1000048
39. Gao Z, Liu H, Daxinger L, Pontes O, He X, et al. 2010. An RNA Polymerase II- and AGO4-associated protein acts in RNA-directed DNA methylation. *Nature* 465(7294):106–9
40. Gehring M. 2013. Genomic imprinting: insights from plants. *Annu. Rev. Genet.* 47(1):187–208
41. Gendrel A, Heard E. 2014. Noncoding RNAs and epigenetic mechanisms during X-chromosome inactivation. *Annu. Rev. Cell Dev. Biol.* 30:561–80
42. Gent JI, Ellis NA, Guo L, Harkess AE, Yao Y, et al. 2013. CHH islands: *de novo* DNA methylation in near-gene chromatin regulation in maize. *Genome Res.* 23(4):628–37
43. Geoghegan JL, Spencer HG. 2013. Exploring epiallele stability in a population-epigenetic model. *Theor. Popul. Biol.* 83:136–44
44. Geoghegan JL, Spencer HG. 2013. The adaptive invasion of epialleles in a heterogeneous environment. *Theor. Popul. Biol.* 88:1–8
45. Geoghegan JL, Spencer HG. 2013. The evolutionary potential of paramutation: a population-epigenetic model. *Theor. Popul. Biol.* 88:9–19
46. Gilly A, Etcheverry M, Madoui M-A, Guy J, Quadrana L, et al. 2014. TE-tracker: systematic identification of transposition events through whole-genome resequencing. *BMC Bioinform.* 15(1):377
47. Hagmann J, Becker C, Müller J, Stegle O, Meyer RC, et al. 2015. Century-scale methylome stability in a recently diverged *Arabidopsis thaliana* lineage. *PLOS Genet.* 11(1):e1004920
48. Heard E, Martienssen RA. 2014. Transgenerational epigenetic inheritance: myths and mechanisms. *Cell* 157(1):95–109
49. Holeski LM, Jander G, Agrawal AA. 2012. Transgenerational defense induction and epigenetic inheritance in plants. *Trends Ecol. Evol.* 27(11):618–26
50. Holliday R. 1987. The inheritance of epigenetic defects. *Science* 238(11):163–70
51. Hollister JD, Gaut BS. 2009. Epigenetic silencing of transposable elements: a trade-off between reduced transposition and deleterious effects on neighboring gene expression. *Genome Res.* 19(8):1419–28
52. Hsieh T-F, Ibarra CA, Silva P, Zemach A, Eshed-Williams L, et al. 2009. Genome-wide demethylation of *Arabidopsis* endosperm. *Science* 324(5933):1451–54
53. Ibarra CA, Feng X, Schoft VK, Hsieh T-F, Uzawa R, et al. 2012. Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. *Science*. 337(6100):1360–64
54. Inagaki S, Miura-Kamio A, Nakamura Y, Lu F, Cui X, et al. 2010. Autocatalytic differentiation of epigenetic modifications within the *Arabidopsis* genome. *EMBO J.* 29(20):3496–3506
55. Ito T, Tarutani Y, To TK, Kassam M, Duvernois-Berthet E, et al. 2015. Genome-wide negative feedback drives transgenerational DNA methylation dynamics in *Arabidopsis*. *PLOS Genet.* 11(4):e1005154
56. Iwasaki M, Paszkowski J. 2014. Identification of genes preventing transgenerational transmission of stress-induced epigenetic states. *PNAS* 111(23):8547–52
57. Jablonka E, Lamb MJ. 1989. The inheritance of acquired epigenetic variations. *Int. J. Epidemiol.* 44(4):1094–103
58. Jiang C, Mithani A, Belfield EJ, Mott R, Hurst LD, Harberd NP. 2014. Environmentally responsive genome-wide accumulation of *de novo Arabidopsis thaliana* mutations and epimutations. *Genome Res.* 24:1821–29
59. Johannes F, Colomé-Tatché M. 2011. Concerning epigenetics and inbreeding. *Nat. Rev. Genet.* 12(5):376
60. Johannes F, Colot V, Jansen RC. 2008. Epigenome dynamics: a quantitative genetics perspective. *Nat. Rev. Genet.* 9(11):883–90
61. Johannes F, Porcher E, Teixeira FK, Saliba-Colombani V, Albuisson J, et al. 2009. Assessing the impact of transgenerational epigenetic variation on complex traits. *PLOS Genet.* 5(6):e1000530

62. Johnson LM, Du J, Hale CJ, Bischof S, Feng S, et al. 2014. SRA- and SET-domain-containing proteins link RNA polymerase V occupancy to DNA methylation. *Nature* 507(7490):124–28
63. Jullien PE, Susaki D, Yelagandula R, Higashiyama T, Berger F. 2012. DNA methylation dynamics during sexual reproduction in *Arabidopsis thaliana*. *Curr. Biol.* 22(19):1825–30
64. Kankel MW, Ramsey DE, Stokes TL, Flowers SK, Haag JR, et al. 2003. *Arabidopsis MET1* cytosine methyltransferase mutants. *Genetics* 163(3):1109–22
65. Kanno T, Mette MF, Kreil DP, Aufsatz W, Matzke M, Matzke AJM. 2004. Involvement of putative SNF2 chromatin remodeling protein DRD1 in RNA-directed DNA methylation. *Curr. Biol.* 14(9):801–5
66. Kawakatsu T, Huang SC, Jupe F, Sasaki E, Schmitz RJ, et al. 2016. Epigenomic diversity in a global collection of *Arabidopsis thaliana* accessions. *Cell*. 166(2):492–505
67. Kawashima T, Berger F. 2014. Epigenetic reprogramming in plant sexual reproduction. *Nat. Rev. Genet.* 15(9):613–24
68. Kohli RM, Zhang Y. 2013. Tet enzymes, TDG and the dynamics of DNA demethylation. *Nature* 502(7472):472–79
69. Kooke R, Johannes F, Wardenaar R, Becker F, Etcheverry M, et al. 2015. Epigenetic basis of morphological variation and phenotypic plasticity in *Arabidopsis thaliana*. *Plant Cell* 27(2):337–48
70. Kronholm I, Collins S. 2015. Epigenetic mutations can both help and hinder adaptive evolution. *Mol. Ecol.* 25(8):1856–68
71. Latzel V, Allan E, Bortolini Silveira A, Colot V, Fischer M, Bossdorf O. 2013. Epigenetic diversity increases the productivity and stability of plant populations. *Nat. Commun.* 4:2875
72. Law JA, Ausin I, Johnson LM, Vashisht AA, Zhu JK, et al. 2010. A protein complex required for polymerase V transcripts and RNA-directed DNA methylation in *Arabidopsis*. *Curr. Biol.* 20(10):951–56
73. Law JA, Du J, Hale CJ, Feng S, Krajewski K, et al. 2013. Polymerase IV occupancy at RNA-directed DNA methylation sites requires SHH1. *Nature* 498(7454):385–89
74. Law JA, Jacobsen SE. 2010. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat. Rev. Genet.* 11(3):204–20
75. Li CF, Pontes O, El-Shami M, Henderson IR, Bernatavichute YV, et al. 2006. An ARGONAUTE4-containing nuclear processing center colocalized with Cajal bodies in *Arabidopsis thaliana*. *Cell* 126(1):93–106
76. Li Q, Eichten SR, Hermanson PJ, Springer NM. 2014. Inheritance patterns and stability of DNA methylation variation in maize near-isogenic lines. *Genetics* 196(3):667–76
77. Li X, Zhu J, Hu F, Ge S, Ye M, et al. 2012. Single-base resolution maps of cultivated and wild rice methylomes and regulatory roles of DNA methylation in plant gene expression. *BMC Genom.* 13(1):300
78. Lippman Z, Gendrel A-V, Black M, Vaughn MW, Dedhia N, et al. 2004. Role of transposable elements in heterochromatin and epigenetic control. *Nature* 430(6998):471–76
79. Lister R, Malley RCO, Tonti-Filippini J, Gregory BD, Berry CC, et al. 2008. Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*. *Cell* 133:523–36
80. Long Q, Rabanal FA, Meng D, Huber CD, Farlow A, et al. 2013. Massive genomic variation and strong selection in *Arabidopsis thaliana* lines from Sweden. *Nat. Genet.* 45(8):884–90
81. López Sánchez A, Stassen J, Furci L, Smith L, Ton J. 2016. The role of DNA (de)methylation in immune responsiveness of *Arabidopsis*. *Plant J.* In press
82. Luna E, Bruce TJA, Roberts MR, Flors V, Ton J. 2012. Next-generation systemic acquired resistance. *Plant Physiol.* 158(2):844–53
83. Manning K, Tör M, Poole M, Hong Y, Thompson AJ, et al. 2006. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat. Genet.* 38(8):948–52
84. Marí-Ordóñez A, Marchais A, Etcheverry M, Martin A, Colot V, Voinnet O. 2013. Reconstructing *de novo* silencing of an active plant retrotransposon. *Nat. Genet.* 45:1029–39
85. Martienssen RA. 1998. Transposons, DNA methylation and gene control. *Trends Genet.* 14(7):263–64
86. Martienssen RA, Colot V. 2001. DNA methylation and epigenetic inheritance in plants and filamentous fungi. *Science* 293(5532):1070–74

87. Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, et al. 2009. A transposon-induced epigenetic change leads to sex determination in melon. *Nature* 461(7267):1135–38
88. Mathieu O, Reinders J, Caikovski M, Smathajitt C, Paszkowski J, et al. 2007. Transgenerational stability of the *Arabidopsis* epigenome is coordinated by CG methylation. *Cell* 130(5):851–62
89. Matzke MA, Mosher RA. 2014. RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. *Nat. Rev. Genet.* 15(6):394–408
90. McClintock B. 1965. The control of gene action in maize. *Brookhaven Symp. Biol.* 18:162–84
91. McCue AD, Nuthikattu S, Reeder SH, Slotkin RK. 2012. Gene expression and stress response mediated by the epigenetic regulation of a transposable element small RNA. *PLOS Genet.* 8(2):e1002474
92. McCue AD, Panda K, Nuthikattu S, Choudury SG, Thomas EN, Slotkin RK. 2015. ARGONAUTE 6 bridges transposable element mRNA-derived siRNAs to the establishment of DNA methylation. *EMBO J.* 34(1):20–35
93. Meng D, Dubin M, Zhang P, Osborne EJ, Stegle O, et al. 2016. Limited contribution of DNA methylation variation to expression regulation in *Arabidopsis thaliana*. *PLOS Genet.* 12(7):e1006141
94. Mirouze M, Reinders J, Bucher E, Nishimura T, Schneeberger K, et al. 2009. Selective epigenetic control of retrotransposition in *Arabidopsis*. *Nature* 461(7262):427–30
95. Miura A, Yonebayashi S, Watanabe K, Toyama T, Shimada H, Kakutani T. 2001. Mobilization of transposons by a mutation abolishing full DNA methylation in *Arabidopsis*. *Nature* 411(6834):212–14
96. Miura K, Agetsuma M, Kitano H, Yoshimura A, Matsuoka M, et al. 2009. A metastable *DWARF1* epigenetic mutant affecting plant stature in rice. *PNAS* 106(27):11218–23
97. Mosher RA, Melnyk CW, Kelly KA, Dunn RM, Studholme DJ, Baulcombe DC. 2009. Uniparental expression of PolIV-dependent siRNAs in developing endosperm of *Arabidopsis*. *Nature* 460(7252):283–86
98. Myant K, Stancheva I. 2008. LSH cooperates with DNA methyltransferases to repress transcription. *Mol. Cell. Biol.* 28(1):215–26
99. Niederhuth CE, Bewick AJ, Ji L, Alabady MS, Do Kim K, et al. 2015. Widespread natural variation of DNA methylation within angiosperms. *bioRxiv* doi: **http://dx.doi.org/10.1101/045880**
100. Nuthikattu S, McCue AD, Panda K, Fultz D, DeFraia C, et al. 2013. The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21–22 nucleotide small interfering RNAs. *Plant Physiol.* 162(1):116–31
101. Ong-Abdullah M, Ordway JM, Jiang N, Ooi S-E, Kok S, et al. 2015. Loss of *Karma* transposon methylation underlies the mantled somaclonal variant of oil palm. *Nature* 525(7570):533–37
102. Ossowski S, Schneeberger K, Lucas-Lledó JI, Warthmann N, Clark RM, et al. 2010. The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. *Science* 327(5961):92–94
103. Pál C. 1998. Plasticity, memory and the adaptive landscape of the genotype. *Proc. R. Soc. B* 265(1403):1319–23
104. Pál C, Miklós I. 1999. Epigenetic inheritance, genetic assimilation and speciation. *J. Theor. Biol.* 200:19–37
105. Penterman J, Zilberman D, Huh JH, Ballinger T, Henikoff S, Fischer RL. 2007. DNA demethylation in the *Arabidopsis* genome. *PNAS* 104(16):6752–57
106. Pontier D, Picart C, Roudier F, Garcia D, Lahmy S, et al. 2012. NERD, a plant-specific GW protein, defines an additional RNAi-dependent chromatin-based pathway in *Arabidopsis*. *Mol. Cell* 48(1):121–32
107. Quadrana L, Almeida J, Asís R, Duffy T, Dominguez PG, et al. 2014. Natural occurring epialleles determine vitamin E accumulation in tomato fruits. *Nat. Commun.* 5:3027
108. Quadrana L, Silveira AB, Mayhew GF, Leblanc C, Martienssen RA, et al. 2016. The *Arabidopsis thaliana* mobilome and its impact at the species level. *eLife*. 5:1–25
109. Regulski M, Lu Z, Kendall J, Donoghue MTA, Reinders J, et al. 2013. The maize methylome influences mRNA splice sites and reveals widespread paramutation-like switches guided by small RNA. *Genome Res.* 23(10):1651–62
110. Reinders J, Wulff BBH, Mirouze M, Mari-Ordoñez A, Dapp M, et al. 2009. Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Dev.* 23(8):939–50

111. Richards EJ. 2006. Inherited epigenetic variation—revisiting soft inheritance. *Nat. Rev. Genet.* 7(5):395–401
112. Riddle NC, Richards EJ. 2002. The control of natural variation in cytosine methylation in *Arabidopsis*. *Genetics* 162(1):355–63
113. Rigal M, Becker C, Pélissier T, Pogorelcnik R, Devos J, et al. 2016. Epigenome confrontation triggers immediate reprogramming of DNA methylation and transposon silencing in *Arabidopsis thaliana* F1 epihybrids. *PNAS* 113(14):E2083–92
114. Rigal M, Kevei Z, Pélissier T, Mathieu O. 2012. DNA methylation in an intron of the *IBM1* histone demethylase gene stabilizes chromatin modification patterns. *EMBO J.* 31(13):2981–93
115. Riggs AD. 1975. X inactivation, differentiation, and DNA methylation. *Cytogenet. Genome Res.* 14(1):9–25
116. Roux F, Colomé-Tatché M, Edelist C, Wardenaar R, Guerche P, et al. 2011. Genome-wide epigenetic perturbation jump-starts patterns of heritable variation found in nature. *Genetics* 188(4):1015–17
117. Saze H, Shiraishi A, Miura A, Kakutani T. 2008. Control of genic DNA methylation by a jmjC domain-containing protein in *Arabidopsis thaliana*. *Science* 319(5862):462–65
118. Schmitz RJ, He Y, Valdes-Lopez O, Khan SM, Joshi T, et al. 2013. Epigenome-wide inheritance of cytosine methylation variants in a recombinant inbred population. *Genome Res.* 23:1663–74
119. Schmitz RJ, Schultz MD, Lewsey MG, O'Malley RC, Urich MA, et al. 2011. Transgenerational epigenetic instability is a source of novel methylation variants. *Science* 334(6054):369–73
120. Schmitz RJ, Schultz MD, Urich MA, Nery JR, Pelizzola M, et al. 2013. Patterns of population epigenomic diversity. *Nature* 495(7440):193–98
121. Secco D, Wang C, Shou H, Schultz MD, Chiarenza S, et al. 2015. Stress induced gene expression drives transient DNA methylation changes at adjacent repetitive elements. *eLife* 4:1–26
122. Seisenberger S, Peat JR, Hore TA, Santos F, Dean W, Reik W. 2013. Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. *Philos. Trans. R. Soc. B* 368(1609):20110330
123. Seymour DK, Koenig D, Hagmann J, Becker C, Weigel D. 2014. Evolution of DNA methylation patterns in the Brassicaceae is driven by differences in genome organization. *PLOS Genet.* 10(11):e1004785
124. Shen X, De Jonge J, Forsberg SKG, Pettersson ME, Sheng Z, et al. 2014. Natural *CMT2* variation is associated with genome-wide methylation changes and temperature seasonality. *PLOS Genet.* 10(12):e1004842
125. Silveira AB, Trontin C, Cortijo S, Barau J, Del Bem LEV, et al. 2013. Extensive natural epigenetic variation at a de novo originated gene. *PLOS Genet.* 9(4):e1003437
126. Singer T, Yordan C, Martienssen RA. 2001. Robertson's *Mutator* transposons in *A. thaliana* are regulated by the chromatin-remodeling gene *Decrease in DNA Methylation* (*DDM1*). *Genes Dev.* 15(5):591–602
127. Slotkin RK, Vaughn M, Borges F, Tanurdžić M, Becker JD, et al. 2009. Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* 136(3):461–72
128. Stelpflug SC, Eichten SR, Hermanson PJ, Springer NM, Kaeppler SM. 2014. Consistent and heritable alterations of DNA methylation are induced by tissue culture in maize. *Genetics* 198(1):209–18
129. Stroud H, Ding B, Simon SA, Feng S, Bellizzi M, et al. 2013. Plants regenerated from tissue culture contain stable epigenome changes in rice. *eLife* 2:e00354
130. Stroud H, Do T, Du J, Zhong X, Feng S, et al. 2014. Non-CG methylation patterns shape the epigenetic landscape in *Arabidopsis*. *Nat. Struct. Mol. Biol.* 21(1):64–72
131. Stroud H, Greenberg MVC, Feng S, Bernatavichute YV, Jacobsen SE. 2013. Comprehensive analysis of silencing mutants reveals complex regulation of the *Arabidopsis* methylome. *Cell* 152(1–2):352–64
132. Takuno S, Gaut BS. 2013. Gene body methylation is conserved between plant orthologs and is of evolutionary consequence. *PNAS* 110(5):1797–802
133. Takuno S, Ran J-H, Gaut BS. 2016. Evolutionary patterns of genic DNA methylation vary across land plants. *Nat. Plants* 2:15222
134. Tanurdzic M, Vaughn MW, Jiang H, Lee T-J, Slotkin RK, et al. 2008. Epigenomic consequences of immortalized plant cell suspension culture. *PLOS Biol.* 6(12):2880–95
135. Teixeira FK, Colot V. 2009. Gene body DNA methylation in plants: A means to an end or an end to a means? *EMBO J.* 28(8):997–98

136. Teixeira FK, Heredia F, Sarazin A, Roudier F, Boccara M, et al. 2009. A role for RNAi in the selective correction of DNA methylation defects. *Science* 323(5921):1600–4
137. Thakore PI, Black JB, Hilton IB, Gersbach CA. 2016. Editing the epigenome: technologies for programmable transcription and epigenetic modulation. *Nat. Methods* 13(2):127–37
138. Tsukahara S, Kobayashi A, Kawabe A, Mathieu O, Miura A, Kakutani T. 2009. Bursts of retrotransposition reproduced in *Arabidopsis*. *Nature* 461(7262):423–26
139. van der Graaf A, Wardenaar R, Neumann DA, Taudt A, Shaw RG, et al. 2015. Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. *PNAS* 112(21):6676–81
140. Vaughn MW, Tanurdzić M, Lippman Z, Jiang H, Carrasquillo R, et al. 2007. Epigenetic natural variation in *Arabidopsis thaliana*. *PLOS Biol.* 5(7):e174
141. Verhoeven KJF, van Gurp TP. 2012. Transgenerational effects of stress exposure on offspring phenotypes in apomictic dandelion. *PLOS ONE* 7(6):e38605
142. Vining KJ, Pomraning KR, Wilhelm LJ, Priest HD, Pellegrini M, et al. 2012. Dynamic DNA cytosine methylation in the *Populus trichocarpa* genome: tissue-level variation and relationship to gene expression. *BMC Genom.* 13:27
143. Vojta A, Dobrinić P, Tadić V, Bočkor L, Korać P, et al. 2016. Repurposing the CRISPR-Cas9 system for targeted DNA methylation. *Nucleic Acids Res.* 44(12):5615–28
144. Vongs A, Kakutani T, Martienssen RA, Richards EJ. 1993. *Arabidopsis thaliana* DNA methylation mutants. *Science* 260(5116):1926–28
145. Waddington CH. 1942. Canalization of development and the inheritance of acquired characters. *Nature* 3811:563–65
146. Weigel D, Colot V. 2012. Epialleles in plant evolution. *Genome Biol.* 13(10):249
147. Weigel D, Nordborg M. 2015. Population genomics for understanding adaptation in wild plant species. *Annu. Rev. Genet.* 49(1):315–38
148. Wibowo A, Becker C, Marconi G, Durr J, Price J, et al. 2016. Hyperosmotic stress memory in *Arabidopsis* is mediated by distinct epigenetically labile sites in the genome and is restricted in the male germline by DNA glycosylase activity. *eLife*. 5:e13546
149. Willing E-M, Rawat V, Mandáková T, Maumus F, James GV, et al. 2015. Genome expansion of *Arabis alpina* linked with retrotransposition and reduced symmetric DNA methylation. *Nat. Plants* 1:14023
150. Woo H, Richards EJ. 2008. Natural variation in DNA methylation in ribosomal RNA genes of *Arabidopsis thaliana*. *BMC Plant Biol.* 8(1):1–12
151. Yang D-L, Zhang G, Tang K, Li J, Yang L, et al. 2016. Dicer-independent RNA-directed DNA methylation in *Arabidopsis*. *Cell Res.* 26(1):66–82
152. Ye R, Chen Z, Lian B, Rowley MJ, Xia N, et al. 2015. A Dicer-independent route for biogenesis of siRNAs that direct DNA methylation in *Arabidopsis*. *Mol. Cell* 61(2):222–35
153. Yi H, Riddle NC, Stokes TL, Woo HR, Richards EJ. 2004. Induced and natural epigenetic variation. *Cold Spring Harb. Symp. Quant. Biol.* 69:155–60
154. Yu A, Lepère G, Jay F, Wang J, Bapaume L, et al. 2013. Dynamics and biological relevance of DNA demethylation in *Arabidopsis* antibacterial defense. *PNAS* 110(6):2389–94
155. Zemach A, Kim MY, Hsieh PH, Coleman-Derr D, Eshed-Williams L, et al. 2013. The *Arabidopsis* nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. *Cell* 153(1):193–205
156. Zemach A, McDaniel IE, Silva P, Zilberman D. 2010. Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science* 328(5980):916–19
157. Zhai J, Bischof S, Wang H, Feng S, Lee T, et al. 2015. A one precursor one siRNA model for Pol IV-dependent siRNA biogenesis. *Cell* 163(2):445–55
158. Zhang X, Yazaki J, Sundaresan A, Cokus S, Chan SW-L, et al. 2006. Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. *Cell* 126(6):1189–201
159. Zhang Y-Y, Fischer M, Colot V, Bossdorf O. 2013. Epigenetic variation creates potential for evolution of plant phenotypic plasticity. *New Phytol.* 197(1):314–22
160. Zheng B, Wang Z, Li S, Yu B, Liu JY, Chen X. 2009. Intergenic transcription by RNA polymerase II coordinates Pol IV and Pol V in siRNA-directed transcriptional gene silencing in *Arabidopsis*. *Genes Dev.* 23(24):2850–60

161. Zhong S, Fei Z, Chen Y, Zheng Y, Huang M, et al. 2013. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. *Nat. Biotechnol.* 31(2):154–59
162. Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S. 2006. Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. *Nat. Genet.* 39(1):61–69
163. Zhong X, Du J, Hale CJ, Gallego-Bartolome J, Feng S, et al. 2014. Molecular mechanism of action of plant DRM de novo DNA methyltransferases. *Cell.* 157(5):1050–60

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Errata

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