EPIGENETICS

Exploiting induced and natural epigenetic variation for crop improvement

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Abstract | Plant breeding has traditionally relied on combining the genetic diversity present within a species to develop combinations of alleles that provide desired traits. Epigenetic diversity may provide additional sources of variation within a species that could be captured or created for crop improvement. It will be important to understand the sources of epigenetic variation and the stability of newly formed epigenetic variants over generations to fully use the potential of epigenetic variation to improve crops. The development and application of methods for widespread epigenome profiling and engineering may generate new avenues for using the full potential of epigenetics in crop improvement.

Traits
Any measurable aspect of an organism, including morphological, biochemical and molecular properties.

Transgressive segregation
The situation in which offspring (F_1, F_2 or later generations) exhibit phenotypes that transgress (are outside of) the parental phenotypic range.

Heritable variation
Information in the genome that is transmitted to offspring or daughter cells.

Genomic selection
The use of genetic markers that are spread throughout the genome to select individuals with desired predicted breeding values.

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Agriculture has a key role in human society. We depend on domesticated plant species such as rice, wheat, soybean and maize to provide fuel, food and fibre resources. We face growing pressure to increase the sustainable productivity of agricultural systems. The human population continues to expand rapidly, and agricultural productivity will need to increase while also limiting potential adverse environmental consequences[1,2]. Humans have been carrying out artificial selection on plants for thousands of years to influence various traits that shape plants for better growth and performance in cultivated environments.

Historically, the bulk of breeding processes relied on transgressive segregation. Two varieties would be crossed and breeders would select offspring that exhibited superior performance relative to the two parents. This process has been repeated multiple times to produce the current elite varieties for most crops. In many cases, the ideal characteristics have changed with increasing mechanization and altered agronomic practices. The modern elite varieties of crop species have been selected to contain many favourable alleles that increase yield in current field environments. Notably, this process relied on the selection of traits and was not dependent on any molecular mechanism of inheritance. This allowed breeding and selection to act on all types of heritable variation present in plant genomes.

In recent years, new tools and approaches for plant improvement have emerged. The availability of cheap assays for genotyping plant materials has enabled genomic selection, the large-scale prediction of traits based on DNA markers for crop improvement[3]. In addition, new traits can be introduced through the addition or editing of genetic information, for example, by using RNA-guided endonucleases such as the CRISPR–Cas9 system. These approaches have focused on improving crops through changes in nucleotide sequence between individuals[4].

Although genomic selection and other molecular marker-based selection approaches that are currently used for plant breeding rely on monitoring genetic variation, there is growing evidence that epigenetics (BOX 1) also has the potential to contribute to important traits in many plant species. Epigenetics — defined in this Review as an inherited change in a phenotype that is not solely due to a change in DNA sequences — can have important roles in creating variation that is inherited by offspring and may not be adequately surveyed in current genomic selection platforms. Epigenetic phenomena such as paramutation, transgenic silencing, imprinting and transposable element inactivation are prevalent in plants, and plant species have provided useful model systems to study the mechanistic bases of these observations[5]. However, the potential for applying epigenetics to crop improvement has received less attention.

There are several important questions that must be addressed to determine the potential avenues for crop improvement through epigenetic approaches. Researchers must understand the level of epigenetic variation within species and how factors such as the external environment or the internal genomic environment influence epigenetic variation. The process of crossing and selection followed by widespread propagation of optimized varieties requires understanding of the stability of epigenetic information

Box 1 | **Defining epigenetics and epigenomics**

The term epigenetics has been defined in a number of different ways[4]. Epigenetics was originally coined by Waddington[132] to describe how the same genetic information is used differently in developmentally distinct tissues. Over time, many researchers came to use this term to describe examples of inheritance that could not be defined by Mendelian or quantitative genetics, such as imprinting, X-chromosome inactivation, paramutation and transgene silencing[4]. As molecular genetic approaches have revealed differences in chromatin, such as altered DNA methylation and histone modification, underlying the silenced state for many of these examples of unusual inheritance, some researchers have come to define these chromatin modifications themselves as epigenetic. There are currently two distinct classes of definitions of epigenetics that are widely used. One set of definitions is based on genetic behaviour and inheritance. These definitions seek to use the term epigenetics to describe inherited (mitotic or meiotic) changes in gene expression that do not involve changes in DNA sequence. The other class of definitions is based on biochemical properties of chromatin and defines any change in chromatin as being epigenetic. Both uses of the term epigenetic have value, but readers are cautioned to make sure that they understand how the term is being used in various publications to interpret whether the authors are describing biochemical or genetic properties of a system. We prefer to use the term epigenetics to describe the genetic behaviour of a system and to use the term chromatin modifications to describe the changes in the biochemical properties of chromatin. Epigenomics is used to describe the genome-wide maps of chromatin, which can include genomic profiles of chromatin accessibility, histone variants, histone modifications and DNA methylation. The epigenome is the collection of chromatin patterns in a particular cell and can include both heritable and transient information.

across multiple generations. How certain common practices in plant breeding and agriculture, such as wide crosses, clonal propagation and tissue culture, may influence epigenetic variation must also be considered. New technologies are also providing opportunities to consider reshaping plant epigenomes to create useful traits. In this Review, we discuss methods and approaches for using epigenetic variation to develop improved plant varieties.

Molecular mechanisms of epigenetics

A self-perpetuating signal is the core of most heritable epigenetic phenomena, and currently the most well understood epigenetic phenomena are linked to stably inherited changes in gene expression. The molecular mechanisms that contribute to epigenetic phenomena, such as histone modifications and DNA methylation, often involve self-reinforcing loops. These loops form the basis of the developmental programming of gene expression, and propagation of these loops across generations forms the basis of epigenetic inheritance[6]. Understanding how these loops are initially established and how they are maintained is fundamental to exploiting epigenetics for the improvement of crop species. Several recent reviews[7–13] have provided detailed information on the various molecular mechanisms that contribute to epigenomic information in plants. We provide a brief overview of these mechanisms, with a focus on DNA methylation, which is the epigenetic modification for which the mechanisms of inheritance and of variation within plant populations are known in the greatest detail. Other types of epigenomic information, such as histone variants or histone modifications, can also exhibit some level of stable inheritance through mitosis[14] and probably have key roles in regulating plant responses to environmental conditions and development (BOX 2). However, it is less clear whether these types of epigenomic information are crucial for trait inheritance across generations, which is necessary for plant breeding and improvement.

Types of DNA methylation in plants. In plant genomes, alterations to cytosine DNA methylation are commonly associated with epigenetic phenomena[4]. DNA methylation is a covalent modification of DNA that is inherited on the parent DNA strand through each round of DNA replication. DNA methylation occurs at three sequence contexts in plant genomes: CG, CHG (where H = A, C or T) and CHH (FIG. 1). Several distinct mechanisms ensure that DNA methylation is faithfully inherited through cell divisions (FIG. 1a), and many of the details of these mechanisms have been determined in *Arabidopsis thaliana*[15,16]. Methylation at CG sites occurs through a self-reinforcing loop that relies on the symmetry of CG dinucleotides. Upon each round of DNA replication, the newly synthesized unmethylated strand creates a hemimethylated substrate that leads to the recruitment of the maintenance CG methyltransferase, METHYLTRANSFERASE 1 (MET1), and methylation of the opposing unmethylated CG site[17]. Maintenance of CHG methylation occurs through a distinct self-reinforcing loop that requires the activity of the histone 3 lysine 9 (H3K9) methyltransferases KRYPTONITE (KYP; also known as SUPPRESSOR OF VARIEGATION 3–9 HOMOLOGUE 4 (SUVH4)), SUVH5 and SUVH6, and the CHG methyltransferase CHROMOMETHYLASE 3 (CMT3)[18,19]. These self-reinforcing enzymes bind each other's products and ensure the maintenance of CHG methylation and H3K9me2 at genomic regions. Methylation at CHH sites occurs through at least two distinct mechanisms. CMT2 recognizes H3K9me2 present in long transposable elements that are usually found in heterochromatin, and that in turn induces the methylation of DNA at CHH sites, especially CAA and CTA sites[20]. CHH methylation in euchromatic regions mostly depends on the activities of a self-reinforcing loop that is created by the production of 24-nucleotide small interfering RNAs (siRNAs) that guide a *de novo* methyltransferase, DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), to target sequences[21]. Recent evidence has suggested that it might be important to consider trinucleotide sequence context beyond CHH and CHG (BOX 3). These maintenance pathways dominate activities in the epigenome; however, there are pathways that have evolved to silence previously untargeted sequences. Recent studies have highlighted the potential roles of pathways that use 21-nucleotide small RNAs and RDR6 (REFS 6,22–26) in triggering the *de novo* methylation of previously unmethylated sequences. Although flowering plant genomes dedicate substantial efforts to ensuring the maintenance of DNA methylation, understanding how it is initially established is important for efforts to engineer epigenomes to improve crop performance.

Genomic distribution of DNA methylation. *A. thaliana* has been a useful model system to explore the mechanisms of establishing, maintaining and removing DNA

Paramutation
An interaction between alleles in which one alleles triggers a heritable change at the other allele, resulting in altered expression or chromatin state.

Imprinting
Differential expression of alleles depending on parent-of-origin of the allele.

Wide crosses
Crosses between very distantly related members of the same species or between individuals of related species.

RNA-directed DNA methylation
(RdDM). The mechanism by which 24-nt small interfering RNAs can direct DNA methylation to specific genomic loci.

Accessions
Individuals isolated from a single geographical area. An ecotype comprises many accessions from a similar ecological range.

Differentially methylated regions
(DMRs). Genomic regions that have different levels of methylation between sample groups. Can be context specific (CG, CHG or CHH) or can refer to overall methylcytosine content.

methylation in plant genomes; however, as descriptions of the DNA methylome for other plant species have emerged, variation in these mechanisms has been revealed. Genomic regions can be classified into different domains based on the context-specific patterns of DNA methylation (FIG. 1b). These include regions with substantial methylation in all three contexts (CG, CHG and CHH) that probably result from the ongoing targeting of methylation to this region through RNA-directed DNA methylation (RdDM) and/or CMT2 (REFS 16,27,28). There are also regions with CG and CHG methylation that do not have CHH methylation, probably as a result of active maintenance by MET1 and CMT3 (REF. 16). CG-only methylated regions of the genome probably result from the activity of MET1 (REF. 29). Many of these CG-only methylated regions occur within the central portions of coding sequences and are known as gene body methylation (gbM)[30]. The remaining portions of the genome can be classified as unmethylated or as having intermediate levels of methylation (unclassified). The relative frequency of these types of methylation domains substantially varies among different plant species[31,32] and also exhibits substantial variation along the length of the chromosome (FIG. 1c). The functional consequences of these types of methylation on gene expression often depend on the location of the methylation relative to the gene. Many genes can tolerate substantial levels of methylation in flanking regions[33]. The presence of gbM, CG-only methylation in gene bodies, seems to have fairly minimal, if any, effects on gene expression[34,35]. This type of methylation is also associated with moderately expressed genes that are slowly evolving, long (in terms of base pairs) and often over-represented in 'housekeeping' functions[36]; and the function of gbM in influencing gene expression variation or specific phenotypic traits is still unknown. By contrast, the presence of any type of methylation (including CG-only methylation) over the transcription start site is often associated with gene silencing[31].

Natural and induced epigenetic variation

DNA methylation would need to exhibit substantial natural variation and be able to influence important plant traits to have an important role in traditional approaches to plant breeding and improvement. Scans of diverse accessions of *A. thaliana*, maize, rice, soybean and *Brachypodium distachyon* have revealed substantial levels of natural variation of DNA methylation profiles[37–47]. In most studies, >99% of the methylome is conserved within a species[44]. However, there are still hundreds to thousands of differentially methylated regions (DMRs) between accessions. Although some of these DMRs reflect subtle differences in gbM that do not seem to be associated with altered gene expression, many of the DMRs between genotypes reflect altered targeting of RdDM or heterochromatin[42–44]. Several studies have also found higher levels of methylation variation in specific accessions that probably reflect functional variation of specific genes involved in the maintenance of epigenetic regulation in these accessions[48–50].

For the natural variation of DNA methylation to affect plant traits it would probably need to alter levels of gene expression[51,52]. For the majority of DMRs, nearby genes do not exhibit changes in expression[40,53]. However, for ~10–20% of DMRs, there is a negative association between methylation and gene expression[40,42,43], suggesting that a subset of methylation variation has the potential to influence phenotype. Genes that exhibit qualitative, on–off expression differences are more likely to be associated with altered levels of DNA methylation than are genes with quantitative differences in expression[44]. These genome-wide analyses of methylomes and transcriptomes highlight the natural variation of DNA methylation and its potential to influence gene expression and plant traits. A number of classical genetic studies identified natural variation attributed to epialleles that affected plant traits such as floral morphology[54], fruit ripening[55] and anthocyanin content[56].

Another line of evidence for the potential role of DNA methylation in influencing quantitative traits in plants is derived from the analysis of epigenetic recombinant inbred line (epiRIL) populations in *A. thaliana*[57,58]. The epiRILs are generated by crossing two genetically identical plants that differ in DNA methylation levels owing to one parent being a homozygous mutant for a gene required for the proper maintenance of DNA methylation. The selection of offspring with the wild-type copy of this gene followed by multiple generations of self-pollination results

Box 2 | Histone modifications and interplay with DNA methylation

Although this Review is primarily focused on DNA methylation, there is abundant evidence for a role of histone modifications in epigenetic regulation. For example, histone H3 lysine 27 trimethylation (H3K27me3) is crucial for the proper control of imprinting in endosperm tissue[133–135]. Moreover, histone modifications have important roles in response to environmental cues in plants[136,137]. Allelic differences in histone modifications are linked to altered gene expression in rice[138]. In addition, map-based cloning of rice yield quantitative trait loci (QTL) identified a histone acetyltransferase that is important for grain weight and plant biomass[139]. Whereas histone modifications are important for changes in gene expression, evidence that they are stably transmitted and have important roles in heritable epigenetic regulation is limited. Several studies have provided evidence for short-term memory (lasting for 7–10 days) of environmental stress associated with histone modifications[140–142], but evidence for the transmission of these states to offspring is sparse.

Histone modifications clearly have important roles in gene regulation in plants. A key question is whether these histone modifications represent heritable information that could be stably passed to offspring for crop improvement. Some histone modifications participate in feedforward loops along with DNA methylation, which could provide a mechanism for stable inheritance across generations. H3K9me2 and CHG (where H = A, C or T) methylation work together in a self-reinforcing loop to propagate an epigenetic state in plants[28,143]. There is also evidence that DNA methylation and certain histone modifications can act antagonistically in plants. For example, in *Arabidopsis thaliana*, the loss of DNA methylation in the endosperm or in the mutant *met1* leads to the accumulation of H3K27me3 and to the repression of gene expression by the Polycomb silencing pathway[135,144]. There is also evidence for an interplay between H3K4me3 and the RNA-directed DNA methylation machinery in plants[145]. Evidence that histone modifications alone could provide stable transmission of epialleles in plants remains limited. There is some evidence for limited stability of H3K27me3 in animal systems that lack DNA methylation[146,147]. In addition, several heritable epialleles in rice are associated with differences in histone modifications[135,148], suggesting the potential for stable inheritance across generations. Future studies will be important for documenting the potential for histone modifications to provide the stable inheritance of epigenetic states through mitosis or meiosis in plants.

in a population of individuals with very similar genomes (other than some new transposon insertions) that vary in whether particular chromosomal regions were stripped of methylation in the original mutant methylome. If one assumes that there is cryptic information represented by genes in plant genomes that are silenced by DNA methylation, then epiRILs would be expected to have the potential to express this cryptic information in a portion of the offspring, and these cryptic loci could be mapped if they lead to phenotypic variation[59,60]. Indeed, many quantitative traits, such as flowering time, plant height and response to abiotic stress, are influenced in a heritable manner in epiRILs[58–62], and in some cases these effects have been mapped to genomic regions with altered methylation[59,60]. Importantly, many of the DMRs that are segregating in epiRIL populations are also detected as natural variants among *A. thaliana* accessions[59]. This suggests that the variation uncovered in epiRIL populations may also exist as natural variation that could be acted on by natural or artificial selection. Unfortunately, efforts to create similar populations in other plant species have been limited by the lethality of mutants that have strong effects on the methylome in crops[63–65].

Sources of epigenetic variation

There are numerous routes to the formation of epialleles (FIG. 2). Broadly speaking, epialleles can arise from either non-genetic or genetic sources[66,67]. Non-genetic sources of epialleles include spontaneous epialleles due to the failure to properly maintain methylation states or through the off-target effects of small RNAs. Non-genetic sources of epigenetic variation could also include developmental or environmental factors that trigger directed chromatin changes or that influence the stability of epigenetic states. Genetic sources of epialleles include transposon insertions that alter regional chromatin[68] and structural rearrangements, such as genetically linked or unlinked copy number variation[51,69]. The exposure to these loci in *cis* or in *trans* can trigger changes in methylation at a locus

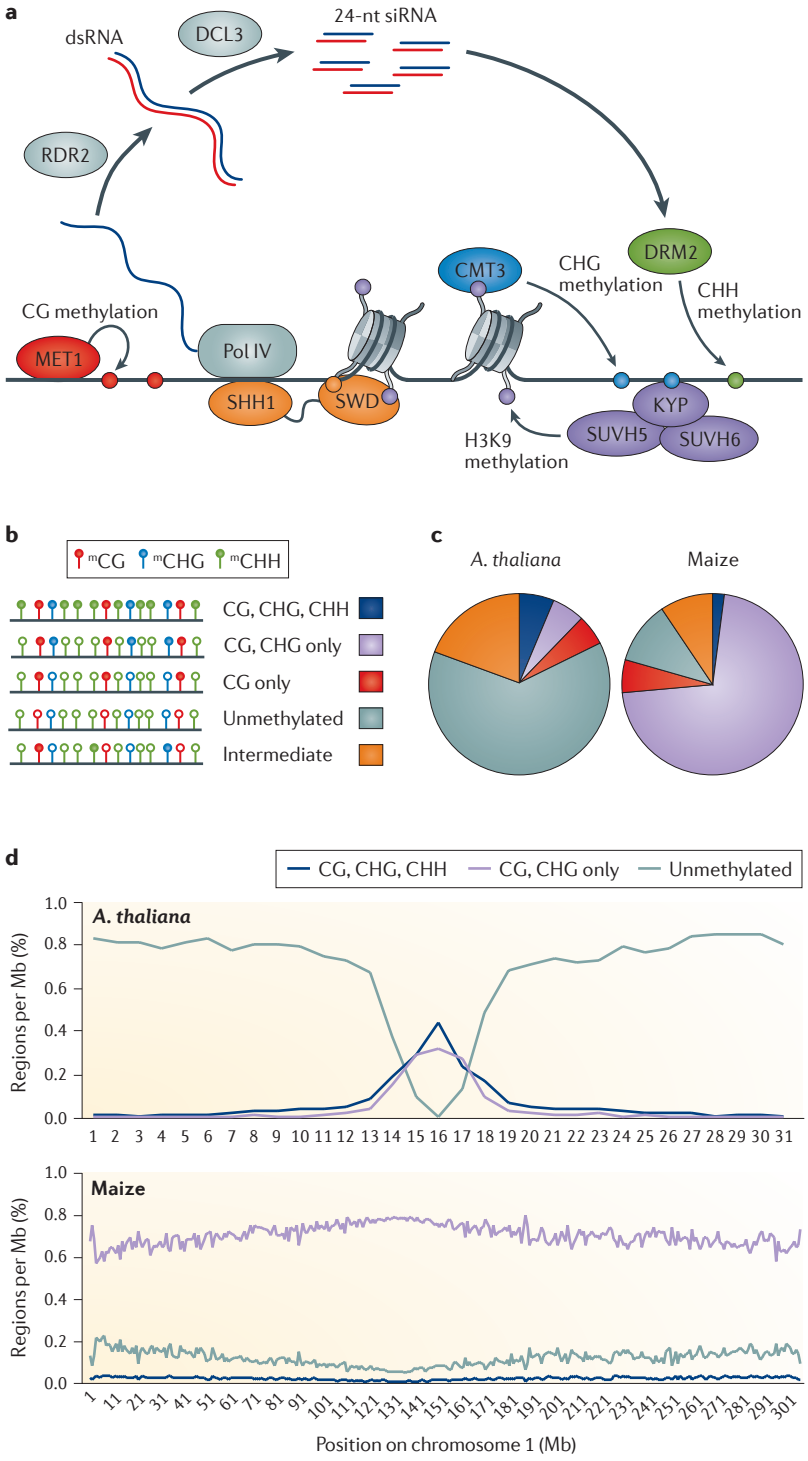
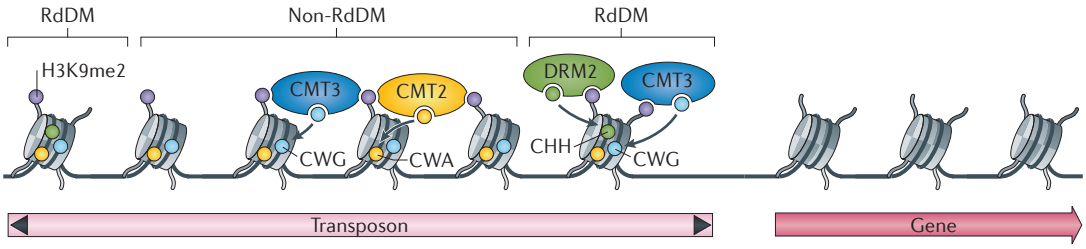


Figure 1 | **Distribution of chromatin domains in plant genomes.** Plants have mechanisms to maintain methylation in CG, CHG (where H = A, C or T) or CHH sequence contexts (part **a**). CG methylation can be maintained following replication by METHYLTRANSFERASE 1 (MET1). CHG methylation can be targeted by a self-reinforcing loop involving the CHG methyltransferase CHROMOMETHYLASE 3 (CMT3) and the histone H3 lysine 9 dimethylation (H3K9me2) methyltransferase KRYPTONITE (KYP). H3K9me2 is also involved in the recruitment of RNA-directed DNA methylation (RdDM) activities, which target DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) and can maintain CHH methylation. Regions of plant genomes can be classified into different domains based on the context-specific levels of DNA methylation. These include CG/CHG/CHH loci (>20% CHH methylation levels), CG/CHG-only loci (>40% CG and CHG), CG-only loci (>40% CG only), unmethylated loci (<10% methylation in all contexts) and loci with intermediate levels (that do not meet any of the above criteria) (part **b**). The relative abundance of these domains can vary substantially in different plant genomes. Maize and *Arabidopsis thaliana* genomes were divided into 100 bp tiles, and each tile was assigned to the first of the categories that it meets (using data from REF. 152) (part **c**). The relative distribution of these CG/CHG/CHH, CG/CHG-only and unmethylated regions is shown for chromosome 1 in *A. thaliana* and maize (part **d**). dsRNA, double-stranded RNA; nt, nucleotide; siRNA, small interfering RNA; SUVH, SUPPRESSOR OF VARIEGATION 3–9 HOMOLOGUE.

Box 3 | **Beyond CHH and CHG**



The study of cytosine methylation in plants uses surrounding nucleotide base composition (sequence context) to understand specific molecular processes, that is, methylation is thought to be maintained in the context of CG, CHG or CHH, which are dependent on different mechanisms. Recent studies have suggested that the reliance on these three contexts has been an oversimplification of context-specific DNA methylation processes in plants[20,149]. Previous work provided clear evidence for the role of chromomethylases such as CHROMOMETHYLASE 3 (CMT3) and ZMET2 in the maintenance of CHG methylation in many plant species[150,151]. However, analyses of all possible trinucleotide contexts show that the primary specificity for these enzymes is actually CWG (where W is A or T)[20]. The remaining CHG site, CCG, has a low level of methylation in plant genomes in comparison to CWG sites. Methylation at the CCG sites may have specific roles in the spreading of gene body methylation[149]. In *Arabidopsis thaliana*, CHH methylation has been attributed to the activities of the DOMAINS REARRANGED METHYLTRANSFERASE (DRM) family targeted by RNA-directed DNA methylation (RdDM) activities, as well as CMT2 (REFS 27,28). It has been suggested that DRM and RdDM primarily function at borders between heterochromatin and euchromatin, whereas CMT2 functions to provide CHH methylation in larger heterochromatic regions[27]. A more careful assessment of the sequence context for methylation suggests that CMT2 is primarily responsible for methylation at CWA sites[20]. This finding suggests that CMT enzymes prefer CWN sites, with CMT3 preferring to methylate CWG sites and CMT2 preferentially methylating CWA sites (see the figure). In maize, which lacks a CMT2 orthologue[27], the *Zmet2* and *Zmet5* gene products seem to be capable of performing both CWG and CWA methylation[65,20]. It remains unknown why CMT enzymes, which are recruited similarly by histone H3 lysine 9 (H3K9) methylation, have specificity for different sites and whether CWA and CWG methylation have differing functional importance in plant genomes.

through various mechanisms, such as the production of siRNAs that would trigger RdDM or the recruitment of heterochromatin. Individuals that are heterozygous for distinct epigenetic states can also undergo paramutation, or directed allelic interactions that influence the formation of epialleles[56]. A crucial issue for our ability to use epigenetic information for crop improvement is in understanding the stability of epigenomic patterns in an organism. If DNA methylation patterns are generally stable through development, then the methylome from any one tissue could be used to accurately describe the epigenetic profile of an individual and to predict traits. By contrast, if DNA methylation is heavily influenced by development and differentiation or by environmental conditions, then the profiles probably report the state of a particular organism rather than reflect the predictive properties for a genotype across space and time.

Epigenomic variation during development. As in most cases of biology, a nuanced view of the stability of DNA methylation among cell types of tissues is required. There are well-documented examples of dynamic alterations to DNA methylation profiles for specific organs and cell types. For example, in the developing endosperm, widespread DNA demethylation can be observed that is associated with the activation of endosperm-specific DNA demethylases[70–72]. This observation is likely to reflect the dynamic changes in methylation that occur in specific nuclei of the male and female gametophytes of plants[73–75]. These DNA methylation changes that occur during reproduction are important for the imprinted regulation of gene expression[76], which is primarily observed in endosperm tissue and could be important for seed size and seed quality traits[77,78]. In addition, the changes in DNA methylation that occur in the sperm nuclei of pollen cells[73,79,80] could be important for the inheritance of DNA methylation and may represent potential targets for influencing DNA methylation variation in crop species. There is also evidence for the cell type-specific transcriptional activation of certain DNA demethylases altering DNA methylomes in tomato fruit ripening[81] and in the nodule development of *Medicago truncatula*[82]. However, comparisons of the methylome of vegetative tissues in *A. thaliana* revealed few major changes[43]. A genome-wide analysis of DNA methylation in six cell types of roots revealed very few changes in CG and CHG methylation, even though there are many gene expression changes among these cell types[83]. One of the cell types, columella, exhibits substantially higher levels of CHH methylation, primarily at sites that have detectable, but low, levels of CHH methylation in other cell types[83]. Thus, altered levels of DNA methylation may be important for specific cell types, but the patterns of CG and CHG methylation seem to be generally stable throughout many vegetative plant tissues.

Epigenomic variation in response to the environment. There are similar complexities in the consideration of DNA methylation variability in response to environmental conditions. Although several studies have

Epialleles
Chromatin differences at a locus between different individuals or cells. Note that an epiallele may be due to genetic differences (at *cis*-genomic or *trans*-genomic locations). Thus, some epialleles may reflect epigenetic variation but others may reflect genetic variation.

Epigenetic recombinant inbred line
(epiRIL). A quasi-homozygous line that is almost identical at the genetic level but that segregates at the DNA methylation level. Produced from an initial cross between two individuals with few DNA sequence differences but with contrasting DNA methylation profiles, followed by 6–8 generations of self-pollination.

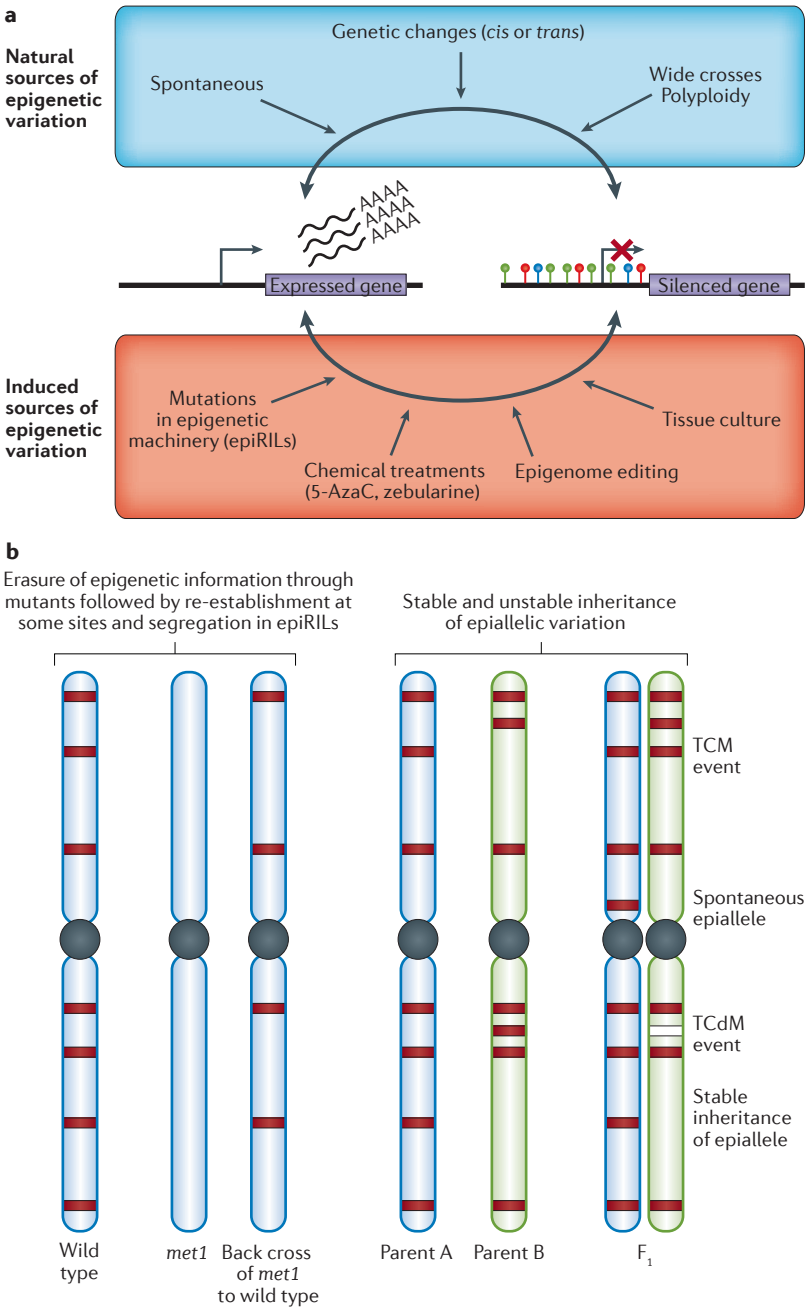


Figure 2 | **Sources of epigenetic variation.** Epialleles that differ for chromatin modifications (such as DNA methylation) and gene expression can arise through various mechanisms (part **a**). Natural sources of epigenetic variation include spontaneous changes, genetic changes in *cis*, such as transposon insertions, structural rearrangements and genetic changes in *trans* that could result in small interfering RNA (siRNA) signals including methylation, interactions among alleles in wide crosses and polyploids. Induced sources of epigenetic variation might include mutations in the epigenetic machinery, such as in the epigenetic recombinant inbred lines (epiRILs), chemical treatments with inhibitors of DNA methylation and other chromatin modifications, directed epigenome editing and treatments with tissue culture or other stresses. The ways in which several of these processes may affect epialleles is shown on plant chromosomes (part **b**). Epialleles can be generated by the passage of a chromosome through a mutant background (such as METHYLTRANSFERASE 1 (*met1*), which erases all CG methylation. When this chromosome is reintroduced to functional MET1 some of these regions are efficiently re-methylated, but others remain unmethylated, providing an opportunity to assess cryptic information in these regions. Natural variation among genotypes (parents A and B) is often stably inherited following crosses. However, at some loci, there can be changes in epialleles state due to *trans*-chromosomal methylation (TCM) or *trans*-chromosomal demethylation (TCdM). 5-AzaC, 5-azacytidine.

reported potential evidence of altered methylation in response to environmental conditions on the basis of methylation-sensitive amplified fragment length polymorphism (MS-AFLP) surveys, there have been some questions about the reproducibility of these studies[84]. A careful study of the methylomes and transcriptomes of *A. thaliana* and rice in response to phosphate starvation revealed almost no changes in *A. thaliana* and ~100 changes in rice[85]. Importantly, many of the DNA methylation changes observed in rice seemed to be specific to CHH sites and were a result, rather than a cause, of altered gene expression[85]. By contrast, several studies have found evidence for altered DNA methylation in response to salt stress in *A. thaliana*[86,87].

There is certainly evidence for a number of DNA methylation changes arising as a result of tissue culture, which represents a very unnatural environment but which is often used for agricultural improvement (FIG. 3). Plants derived from tissue culture exhibit a surprisingly high level of phenotypic variation from the donor material, a phenomenon known as somaclonal variation[88]. Early studies found evidence that tissue culture could reactivate transposons that had been epigenetically silenced, triggering new genetic mutations in plants derived from tissue culture[89,90]. There is also evidence for direct epigenetic changes that influence the expression[91] or the splicing of genes[92] that result from tissue culture. Genome-wide profiling has revealed the hypermethylation of CHH sites in *A. thaliana* cell suspension cultures[93] and rice callus tissue[94]. In rice or maize plants derived from tissue culture there is less evidence for widespread changes in CHH methylation, but several hundred loci exhibit reduced levels of CG and/or CHG methylation that can be stably inherited[94,95]. These studies have provided strong evidence that the tissue culture process can influence epigenetic variation in plants.

Other studies[96,97] have found limited evidence for widespread changes in methylation in response to environmental conditions other than tissue culture. An analysis of *A. thaliana* plants with almost identical genomes that had been growing in naturally varying environments for ~100 years revealed very few differences in DNA methylation profiles[98]. An emerging consensus is that certain stresses may trigger specific changes in methylation at a small number of loci, often associated with transposable elements[99]. However, the bulk of the methylome, especially in CG and CHG sequence contexts, seems to be stable.

The stability of DNA methylation in response to environmental variation is a crucial factor in how researchers would approach the use of epigenetics for agricultural improvement. If widespread reprogramming of methylation occurs in response to the environment and is adaptive, then one could envision using Lamarckian approaches to adapting plants to specific environments. For example, this line of thought would suggest that researchers could improve the cold tolerance of a species simply through the exposure of parental plants to severe cold stress. There is limited evidence for heritable gains in stress tolerance traits owing to environmental exposures in the absence of genetic

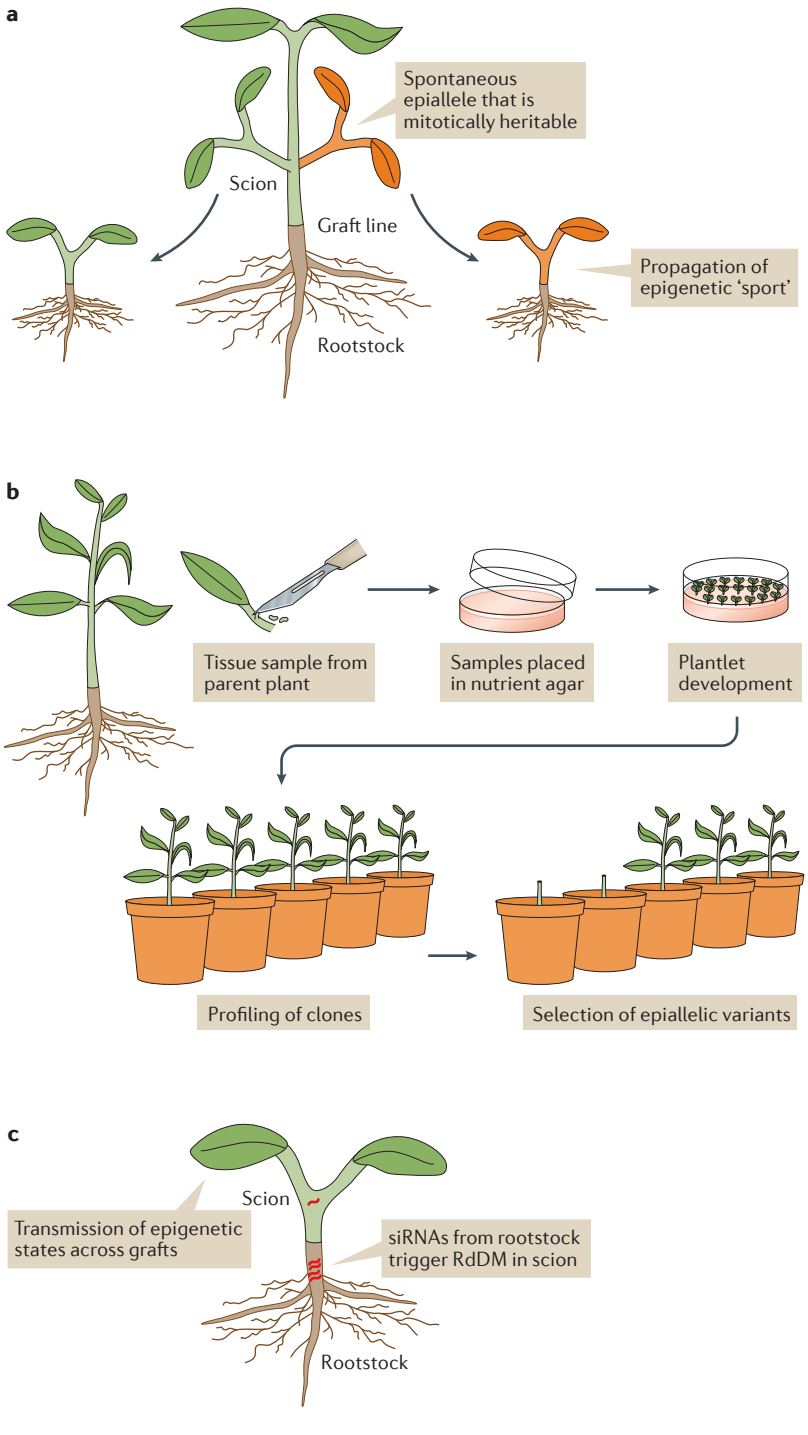


Figure 3 | **Epigenetic variation and clonal propagation.** The vegetative propagation of plants creates the opportunity to generate and harness epigenetic variation. Whether epigenetic variants arise spontaneously or are induced, they can be stably maintained and propagated through grafting or tissue culture if the epialleles are stably maintained through mitosis (part **a**). For plants that are generated by tissue culture, the process of tissue culture can induce a number of epigenetic changes (part **b**). In many agricultural species, tissue culture is a necessary part of plant transformation and clonal propagation. Tissue culture represents an extreme form of environmental stress and a unique developmental trajectory. Profiling of chromatin marks may therefore be necessary to identify beneficial or deleterious epialleles. In grafted plants, the scion tissue may have targeted epigenetic variation owing to the expression of mobile signals such as small interfering RNAs (siRNAs) in the rootstock (part **c**). RdDM, RNA-directed DNA methylation.

variation. Alternatively, if DNA methylation is generally stable in response to variable environments, then selection for epigenetic states could be relevant for large-scale agricultural application, even though plants will be grown in a variety of environments.

Dynamic behaviour of DNA methylation in wide crosses and polyploids. The act of creating populations in itself could provide a source of novel phenotypic variation if the parental epigenomes are sufficiently diverged. Classical genetic studies have provided evidence at a handful of loci for paramutation[100], the directed interactions between alleles that result in altered epigenetic states. Crosses between different *A. thaliana* accessions with distinct epigenomes have provided evidence for paramutation-like phenomena, known as *trans*-chromosomal methylation (TCM) and *trans*-chromosomal demethylation (TCdM) (FIG. 2), in which the chromatin status of one allele influences the status of the other allele[101,102]. These phenomena occur at a subset of loci that exhibit different epigenetic states in the two parents. In the case of TCM there is an observed association with increased 24-nucleotide siRNAs at the affected allele. TCM events can be inherited by subsequent generations, leading to lasting changes in gene expression[101]. In addition to TCM and TCdM, it has also been observed that in some regions of the genome that are methylated in both parents, both alleles become hypermethylated in the hybrid plants[103]. This hypermethylation is dependent on 24-nucleotide siRNAs and the RdDM pathway. In general, the genomic regions affected by TCM, TCdM or hypermethylation represent a minority of methylated regions between accessions, but the frequency of such events could be dependent on the epigenomes being sufficiently diverged.

An extreme example that led to a high frequency of epigenomic variation, known as 'epigenomic shock', was demonstrated in *A. thaliana* after examining the DNA methylome of hybrids between a wild-type parent and a MET1-deficient parent[104,105]. The *met1* mutants exhibited loss of CG methylation, increased transposon expression and increased CHG methylation in some gene bodies. The increase in CHG methylation is attributed to the loss of DNA methylation in an intron of the *IBM1* gene, which encodes the H3K9 demethylase INCREASE IN BONSAI METHYLATION 1. The loss of methylation in the intron of *IBM1* and the subsequent decrease in its expression is exacerbated in the hybrid plants[105]. Furthermore, most of the 2,000 transposons transcriptionally re-activated in the *met1* parent are immune from re-silencing in the hybrids[105]. These results indicate that, when the two genomes (alleles exposed to *met1* or wild-type *MET1*) are initially present in the nucleus, there is an imbalance in heterochromatin that is not immediately re-established even though the machinery is present. The mechanistic basis for this observation is still unknown. However, it is intriguing that *IBM1* has a heterochromatin-like region in its intron, which enables this gene to potentially 'sense'

the surrounding heterochromatin environment. A similar epigenome sensing mechanism has also been proposed for the DNA demethylase REPRESSOR OF TRANSCRIPTIONAL GENE SILENCING 1 (ROS1)[106,107]. Modulation of the strength of silencing at a heterochromatin-like region in the *ROS1* promoter is correlated with the expression levels of this gene. The ability of *IBM1* and *ROS1* to sense heterochromatin is likely to allow them to serve as important integrators that are required for the maintenance of epigenome stability.

Other examples of increased epigenome variation have been observed throughout the process of polyploidization[108]. Whole-genome duplication is a significant contributor to the evolution of plant genomes. The successful doubling of genomes requires proper reinforcement of silenced regions within each set of chromosomes. Whole-genome duplications occur through autopolyploidization (doubling of chromosome) or through allopolyploidization (doubling of chromosome through the hybridization of distinct genotypes). There is extensive literature on the impact on genetic variation during these processes but until recently there were very few studies resulting from the investigation of epigenome dynamics during polyploidization. A study of the epigenomes of rice autopolyploids revealed that tetraploids had increased non-CG methylation class II DNA transposons compared with diploids[109]. This non-CG methylation was also associated with the presence of 24-nucleotide siRNAs, which are associated with the RdDM pathway. A transcriptome study between the diploids and the tetraploids revealed only a couple of hundred differentially expressed genes, many of which also exhibited altered DNA methylation levels[109].

The consequences of epigenome dynamics might not be consistent between autopolyploids and allopolyploids. A preliminary study of DNA methylomes from a variety of allopolyploids in the monkeyflower *Mimulus* spp. indicated that widespread reductions in CHH methylation of transposons were observed immediately after the hybridization of the two genomes[110]. However, the level of CHH methylation is intermediate in newly synthesized allopolyploids and equilibrates to the parental levels in later generations, as assessed by DNA methylome analysis of synthetic and natural allopolyploids produced from the same parental genotypes[110]. Interestingly, symmetric methylation of CG and CHG sites is unchanged even though the transcriptome of one of the parents dominates the other parent. However, a causal link between alterations in CHH methylation and documented dominant transcriptome of one parent has not yet been determined.

There is great potential for creating epigenetic variation at specific loci using hybrids and/or crossing parents with vastly different epigenomes. The ability to create novel populations using the approaches outlined above will result in phenotypic variation. Whether the phenotypes that arise are linked to epigenetic variation and whether the newly created epigenetic states are stably inherited over generations, and agronomically favourable, will be an active area of investigation.

Inheritance of epigenetic variation

The question of stability of DNA methylation inheritance across generations is crucial to deciding the value of epigenomic information for crop improvement. If the inheritance of DNA methylation is very stable, approaching the level of genetic information, then any epialleles will be faithfully inherited and new epialleles will occur only rarely. However, if DNA methylation patterns are unstable then we might expect the rapid formation, or loss, of epialleles within populations.

In a scenario of very stable inheritance of DNA methylation, the epialleles would have high levels of linkage disequilibrium with nearby genetic polymorphisms. Genome-wide association studies (GWAS) or genomic selection approaches based on dense single nucleotide polymorphism (SNP) maps would thus probably tag the majority of DMRs, and these DMRs could be mapped to regions or captured for crop improvement even if the specific causative change was not identified. By contrast, if DMRs are unstable then they will have limited linkage disequilibrium with nearby SNPs and would have additional information that is not effectively tagged by dense SNP maps. The exact level of instability would influence the portion of epialleles that would be effectively surveyed in controlled populations (such as biparental populations of recombinant inbred lines) or in association panels[67]. Partially stable inheritance would probably provide sufficient stability for mapping in populations with limited numbers of generations, such as recombinant inbred lines, but would probably not be suitable for association panels in which higher stabilities would be required. Additional studies documenting the exact stability of DMR inheritance and modelling linkage disequilibrium across generations will be important for our ability to effectively survey the effects of DNA methylation and potential links to genetic variation.

The stability of inheritance of DNA methylation across multiple generations has been evaluated in several different ways. Perhaps the most direct assessment was through the detailed analysis of DNA methylation in a mutation accumulation population in *A. thaliana*[110–113]. A single *A. thaliana* individual was used to found a population that was propagated by single-seed descent for 30 generations[114]. Genomic sequencing of the original parent and the offspring after 30 generations revealed the spontaneous mutation spectrum and rate for *A. thaliana*[115]. Methylome sequencing was used to assess the rate of epimutation[111,112]. Two distinct types of DNA methylation change could be evaluated in these populations: single cytosine changes in methylation and regional changes (that is, DMRs). Although there were only ~20 SNPs per individual following 30 generations, there were thousands of differentially methylated cytosines. However, although methylation at single cytosines exhibited reduced stability relative to the genetic sequence, DMRs were identified only at hundreds of regions following 30 generations, demonstrating

Polyploidization
Whole-genome duplication events that can occur through the doubling of the chromosomes in a single species (autopolyploidization) or through a cross between related species followed by chromosome doubling (allopolyploidization).

Linkage disequilibrium
A measure of whether alleles at two loci coexist in a population in a nonrandom manner. Alleles that are in linkage disequilibrium are found together on the same haplotype more often than would be expected under a random combination of alleles.

the greater stability of regional methylation levels than of individual modifications. There is also variability for the stability of DNA methylation inheritance based on the sequence context[113]. Several recent studies have attempted to address the question of whether environmental conditions influence the stability of inheritance of DNA methylation. Hagmann *et al.*[98] investigated the methylomes of *A. thaliana* plants grown in natural environments for ~100 years and found relatively few differences, suggesting that methylomes can be stably transmitted, even in varying environments, for many generations. However, in *A. thaliana* plants subjected to salt stress[86] and in rice plants subjected to multiple generations of drought stress[116], there is evidence for increased rates of DNA methylation changes, suggesting that severe environmental stress may trigger altered rates of methylation stability. The use of populations with little or no genetic variation has greatly contributed to our understanding of the stability of DNA methylation variants on distinct timescales.

Considerably more natural variation in DNA methylation exists in genotypes that have greater genetic variation. Numerous studies have assessed the inheritance of variable methylation in the presence of segregating genetic information. Genome-wide association scans of DNA methylation in diverse *A. thaliana* accessions reveal that many DMRs have significant associations with local SNPs or small insertions and deletions (indels), suggesting stable inheritance, whereas other DMRs are associated with changes in other genomic regions, suggesting *trans*-acting control[43,45]. An association-based approach revealed that nearly 50% of DMRs in maize exhibit significant associations with local SNPs, suggesting relatively stable inheritance[40].

The analysis of inheritance for DNA methylation in recombinant inbred populations of maize and soybean has revealed generally stable inheritance with rare examples of unexpected patterns[40,42,117]. Several studies have provided evidence for some examples of potential paramutation-like behaviour, or TCM, in which the methylation state of one allele affects the methylation level of the other allele when present together in a heterozygous state[40,101,102]. In reality, it is quite possible that there are various different underlying causes for DMRs, and these are likely to be reflected in varying levels of stability for the inheritance of these DMRs. Some DMRs may be the result of nearby structural variation[45,69,118] and might be fairly faithfully inherited along with the genetic polymorphism. Other DMRs may reflect stochastic changes in epigenetic state, with no accompanying change in DNA sequence, and may exhibit less stable inheritance. A careful analysis of epiRIL populations provides evidence for this range of behaviours[57,119]. The numerous DMRs between the parent plants exhibit a variety of behaviours in the epiRIL population[119]. Many of the DMRs return to wild-type methylation in the F_1 generation and all subsequent generations, suggesting that the DNA methylation can be effectively re-targeted to these regions by genetic information[57]. Some of the remaining DMRs exhibit very stable inheritance and can be used to map which genomic regions experienced demethylation and the impact on phenotypic variation[59]. Other DMRs exhibit partial stability and slowly return to wild-type methylation levels over multiple generations[119]. The range of stability for the behaviour of DMRs may also hold true for natural variation[40,42,103,104,109], and the source of an epiallele can probably influence stability over generations. Epialleles that are the result of genetic variation, such as nearby transposon insertions, may be fairly stable, as the source of information programming the chromatin modification is consistently present at the locus. Other epialleles that are the result of spontaneous variation may be less stable, as there is no source of reinforcement of the epigenetic information.

Epigenetics in clonally propagated species

In most cases, plant breeding efforts have focused on harnessing natural variation through genetic crosses and the evaluation of progeny. This is often followed by the creation of stable inbred or hybrid varieties that can be sold as seeds for agricultural production. However, for other species, we have been forced to use clonal propagation to maintain ideal varieties. Many fruits are the result of clonal propagation and are based on a single genetic variety. In these species, there is the potential for epigenetic variation among different 'sports' or clonal propagants, and epigenetic variation that arises among cells or tissues could be passed on through scions or clonal propagation if it is stable (FIG. 3a). In species that use tissue culture for clonal propagation, the tissue culture itself might also induce novel epigenetic variation (FIG. 3b). Many fruit crops are widely propagated through grafting. In these species, there are opportunities for directed epigenetic changes from rootstock to scion or vice versa[120,121]. In these cases, a rootstock or scion that produces a mobile signal, such as an siRNA, can direct methylation changes across the graft (FIG. 3c). This could be used in agricultural species to target epigenetic changes[122].

The recent study of oil palm points to one avenue for epigenetic information being used for agricultural improvement in clonally propagated species[92]. Most oil palms are hybrids derived from a cross between two subspecies. Once a high-performing cross is identified, it is widely deployed across many plantations by using tissue culture to develop many clones. Unfortunately, a subset of the clones that have passed through tissue culture exhibits a 'mantled' phenotype that destroys the productivity of the tree[92,123]. The mantled trait is not apparent until maturity, years after planting. The analysis of genomic DNA methylation has identified a DMR that is associated with the mantled trait. Changes in DNA methylation of an intron of the *EgDEF1* gene result in aberrant transcripts for a floral identity gene that lead to undesirable morphology changes[92]. Understanding the epigenetic basis for this trait could provide avenues for using DNA methylation profiling to identify the defective clones. For example, some sports of popular apple varieties exhibit unusual colour patterns, and there is evidence for DNA methylation changes at the promoters of transcription factors that are known to regulate anthocyanin production[124]. Maintaining apples with

Scions
Shoot or branch of a plant that is grafted to a rootstock.

Grafting
The joining of living material from two individuals to generate a chimaera. In plants this generally is performed through grafting of a scion (a branch or bud) from one plant to a rootstock from another plant.

Rootstock
The root system of a plant with the shoot removed onto which another variety is grafted.

Epigenetic quantitative trait loci
(epiQTL). Epigenetic variants that are associated with a trait and that do not have any changes in the DNA sequence.

consumer-preferred coloration would require DNA methylation profiles of cuttings. In addition to removing deleterious alleles, there may also be opportunities to unleash epigenetic information in clonally propagated species to generate new beneficial alleles. For example, phenotypic variants in the strawberry were generated through treatment with the methylation inhibitor 5-azacytidine and could be vegetatively propagated[125].

Epigenome engineering for crop improvement

Researchers have uncovered several examples of epialleles that can provide major morphological variation. However, the experimental identification of epialleles that are linked to phenotypic variation is a slow process that often relies on spontaneous epimutations. However, the creation and study of epiRILs using *A. thaliana* radically changed the rate at which epialleles could be generated and associated with phenotypic traits. The epiRIL populations that have been developed display extensive phenotypic variation, ranging from altered disease resistance to variation in biomass[57,58], to name a few examples. The ability to perturb DNA methylomes and then to create individuals with mosaic methylomes reveals that there is a pre-existing untapped source of allelic variation present in plant genomes. Accessing this allelic variation has proved difficult in plants other than *A. thaliana*, as other studied plants are more sensitive to severe genome-wide alterations to DNA methylation[63–65]. Therefore, novel approaches that moderately perturb the DNA methylomes of crop plants and/or methods that are more precise will need to be developed to create novel epiallelic variation in other plant species.

A promising new methodology for engineering DNA methylation states in a site-specific manner in plant genomes uses the fusion of enzymes that can add or remove DNA methylation with proteins that are guided to specific DNA sequences (FIG. 4). Enzymes such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR–Cas9 systems are being rapidly developed to edit genome sequences. Recently, these systems have been used to engineer epigenomes, primarily in mammalian cell lines[126–128]. This has been accomplished by using the same sequence-specific guides and swapping the nucleases with enzymes such as DNA methyltransferases, DNA demethylases or, in the case of CRISPR–Cas9, by fusions with a nuclease-dead form of Cas9 (dCas9)[129]. The first demonstration of site-specific epigenome engineering in plants was accomplished in *A. thaliana* using a ZFN fused to SUVH9, a protein that is integral to RdDM[130]. This fusion could direct DNA methylation to target DNA sequences and caused expected phenotypic consequences. However, it is unclear how widespread the use of this methodology could be, as the reported example relied on a locus that had pre-existing siRNAs and no DNA methylation, which is a rare event in most plant genomes. Regardless, genome-editing technologies are rapidly improving to a point that plant epigenomes will be readily engineered in the not too distant future.

The development of methodologies to site-specifically engineer DNA methylation states in plant genomes will also prove extremely useful for hypothesis testing, as was demonstrated with ZFN–SUVH9 (REF. 130). Currently, the identification of correlations between changes in DNA methylation, gene expression and phenotypic variation is without tests for causality. For crop species that are transformable, the ability to identify causal relationships between differential methylation and the observed phenotypic variations will greatly expand the understanding of the role of DNA methylation in controlling gene expression. This methodology will also facilitate the identification of epigenetic quantitative trait loci (epiQTL) from epiRIL populations and from epigenome-wide association studies. Finally, this methodology could be useful to prevent the silencing of transgenes or even to reactivate silenced transgenes in pre-existing lines. Transgene silencing is a major nuisance to engineering crop genomes, and there is no clear understanding of why transgene silencing occurs. Regardless, developing systems that could survey and demethylate transgene integrations could prove highly valuable.

One of the challenges associated with the site-specific engineering of DNA methylation states is knowing which sequences to target. These epigenome editing tools are useful for hypothesis testing, but are not feasible for engineering DNA methylation states at a seemingly unlimited number of possibilities in crop genomes. One possible way to circumvent this obstacle is to develop methods for epimutagenesis, or widespread random perturbation of DNA methylomes. Methodologies to achieve this include the use of chemical inhibitors of maintenance DNA methylation, such as 5-azacytidine and zebularine[131], as well as the engineering of DNA methyltransferases or DNA demethylases to

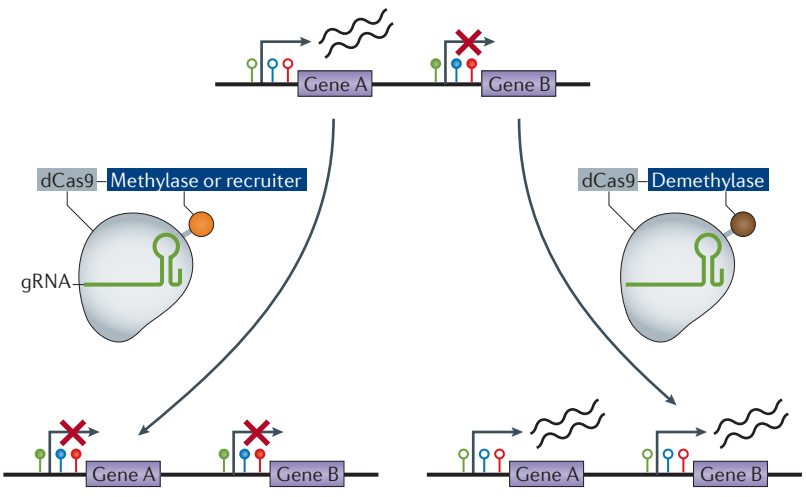


Figure 4 | **Epigenome editing tools.** The genome is a mosaic of methylated and unmethylated regions. CRISPR systems containing a nuclease-dead Cas9 (dCas9) protein fused to a DNA methyltransferase or a protein involved in the recruitment of *de novo* methylation could trigger the methylation of previously unmethylated regions, resulting in reduced gene expression or gene silencing. Alternatively, a targeted dCas9 fusion to a demethylase enzyme could be used to demethylate cryptic genes, resulting in the novel expression of previously methylated genes. gRNA, guide RNA.

act in a genome-wide manner. These approaches may provide new avenues for generating epigenetic diversity in crop species in which loss-of-function mutations in key components of the DNA methylation machinery are inviable[63–65]. The development of epimutagenesis could be especially useful if applied to polyploid genomes, as they have a richer source of allelic variants that are silenced by DNA methylation. Regardless, substantial effort will need to be dedicated to understanding the stability of newly created epialleles between cell divisions and between generations for the widespread adoption of technologies to engineer DNA methylation in crop genomes.

Conclusions

There is a crucial need to improve the efficiency of production of food and fuel supplies for a growing population. Properly harnessing epigenetic variation may provide new opportunities for crop improvement. Technological advances have provided new insights into the sources and inheritance of epigenetic variation. The coming years are likely to see increased opportunities for monitoring and manipulating crop epigenomes. It will be crucial to develop a detailed understanding of how to predict the stability for epigenetic variants such that we can use epigenetics for the stable improvement of agricultural traits.

1. Nelson, G. C. *et al.* Climate change effects on agriculture: economic responses to biophysical shocks. *Proc. Natl Acad. Sci. USA* **111**, 3274–3279 (2014).
2. Garnett, T. *et al.* Agriculture. Sustainable intensification in agriculture: premises and policies. *Science* **341**, 33–34 (2013).
3. Wallace, J. G., Larsson, S. J. & Buckler, E. S. Entering the second century of maize quantitative genetics. *Heredity (Edinb.)* **112**, 30–38 (2014).
4. Ma, X., Zhu, Q., Chen, Y. & Liu, Y. G. CRISPR/Cas9 platforms for genome editing in plants: developments and applications. *Mol. Plant.* **9**, 961–974 (2016).
5. Heard, E. & Martienssen, R. A. Transgenerational epigenetic inheritance: myths and mechanisms. *Cell* **157**, 95–109 (2014).
6. Henikoff, S. & Greally, J. M. Epigenetics, cellular memory and gene regulation. *Curr. Biol.* **26**, R644–R648 (2016).
7. Cuerda-Gil, D. & Slotkin, R. K. Non-canonical RNA-directed DNA methylation. *Nat. Plants* **2**, 16163 (2016).
8. Gutierrez, C., Desvoyes, B., Vergara, Z., Otero, S. & Sequeira-Mendes, J. Links of genome replication, transcriptional silencing and chromatin dynamics. *Curr. Opin. Plant Biol.* **34**, 92–99 (2016).
9. Jiang, D. & Berger, F. Histone variants in plant transcriptional regulation. *Biochim. Biophys. Acta* **1860**, 123–130 (2017).
10. Wendte, J. M. & Pikaard, C. S. The RNAs of RNA-directed DNA methylation. *Biochim. Biophys. Acta* **1860**, 140–148 (2017).
11. Xiao, J., Lee, U. S. & Wagner, D. Tug of war: adding and removing histone lysine methylation in *Arabidopsis*. *Curr. Opin. Plant Biol.* **34**, 41–53 (2016).
12. Vidalis, A. *et al.* Methylome evolution in plants. *Genome Biol.* **17**, 264 (2016).
13. Chen, X. & Zhou, D. X. Rice epigenomics and epigenetics: challenges and opportunities. *Curr. Opin. Plant Biol.* **16**, 164–169 (2013).
14. Gaydos, L. J., Wang, W. & Strome, S. Gene repression. H3K27me and PRC2 transmit a memory of repression across generations and during development. *Science* **345**, 1515–1518 (2014).
15. Law, J. A. & Jacobsen, S. E. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat. Rev. Genet.* **11**, 204–220 (2010).
16. Stroud, H., Greenberg, M. V., Feng, S., Bernatavichute, Y. V. & Jacobsen, S. E. Comprehensive analysis of silencing mutants reveals complex regulation of the *Arabidopsis* methylome. *Cell* **152**, 352–364 (2013).
This detailed analysis reports the contribution of 86 different genes to the distribution of DNA methylation in *A. thaliana*.
17. Bostick, M. *et al.* UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* **317**, 1760–1764 (2007).
18. Lindroth, A. M. *et al.* Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. *Science* **292**, 2077–2080 (2001).
19. Jackson, J. P., Lindroth, A. M., Cao, X. & Jacobsen, S. E. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* **416**, 556–560 (2002).
20. Gouil, Q. & Baulcombe, D. C. DNA methylation signatures of the plant chromomethyltransferases. *PLoS Genet.* **12**, e1006526 (2016).
21. Cao, X. & Jacobsen, S. Locus-specific control of asymmetric and CpNpG methylation by the DRM and CMT3 methyltransferase genes. *Proc. Natl Acad. Sci. USA* **99**, 16491–16498 (2002).
22. Panda, K. *et al.* Full-length autonomous transposable elements are preferentially targeted by expression-dependent forms of RNA-directed DNA methylation. *Genome Biol.* **17**, 170 (2016).
23. Nuthikattu, S. *et al.* The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21–22 nucleotide small interfering RNAs. *Plant Physiol.* **162**, 116–131 (2013).
This study documents the role of specific components in true *de novo* methylation in plants.
24. Bond, D. M. & Baulcombe, D. C. Epigenetic transitions leading to heritable, RNA-mediated *de novo* silencing in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **112**, 917–922 (2015).
25. Wu, L., Mao, L. & Qi, Y. Roles of dicer-like and argonaute proteins in TAS-derived small interfering RNA-triggered DNA methylation. *Plant Physiol.* **160**, 990–999 (2012).
26. Fultz, D. & Slotkin, R. K. Exogenous transposable elements circumvent identity-based silencing, permitting the dissection of expression-dependent silencing. *Plant Cell* **29**, 360–376 (2017).
27. Zemach, A. *et al.* The *Arabidopsis* nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. *Cell* **153**, 193–205 (2013).
28. Stroud, H. *et al.* Non-CG methylation patterns shape the epigenetic landscape in *Arabidopsis*. *Nat. Struct. Mol. Biol.* **21**, 64–72 (2014).
29. Bewick, A. J. & Schmitz, R. J. Gene body DNA methylation in plants. *Curr. Opin. Plant Biol.* **36**, 103–110 (2017).
30. Tran, R. K. *et al.* DNA methylation profiling identifies CG methylation clusters in *Arabidopsis* genes. *Curr. Biol.* **15**, 154–159 (2005).
31. Niederhuth, C. E. *et al.* Widespread natural variation of DNA methylation within angiosperms. *Genome Biol.* **17**, 194 (2016).
This study is a detailed documentation of similarities and differences in methylome patterning in 34 plant species.
32. Takuno, S., Ran, J. H. & Gaut, B. S. Evolutionary patterns of genic DNA methylation vary across land plants. *Nat. Plants* **2**, 15222 (2016).
33. Li, Q. *et al.* RNA-directed DNA methylation enforces boundaries between heterochromatin and euchromatin in the maize genome. *Proc. Natl Acad. Sci. USA* **112**, 14728–14733 (2015).
34. Zhang, X. *et al.* Genome-wide high-resolution mapping and functional analysis of DNA methylation in arabidopsis. *Cell* **126**, 1189–1201 (2006).
35. Bewick, A. J. *et al.* On the origin and evolutionary consequences of gene body DNA methylation. *Proc. Natl Acad. Sci. USA* **113**, 9111–9116 (2016).
36. Takuno, S. & Gaut, B. S. Body-methylated genes in *Arabidopsis thaliana* are functionally important and evolve slowly. *Mol. Biol. Evol.* **29**, 219–227 (2012).
37. Vaughn, M. W. *et al.* Epigenetic natural variation in *Arabidopsis thaliana*. *PLoS Biol.* **5**, e174 (2007).
38. Eichten, S. R. *et al.* Heritable epigenetic variation among maize inbreds. *PLoS Genet.* **7**, e1002372 (2011).
39. Chodavarapu, R. K. *et al.* Transcriptome and methylome interactions in rice hybrids. *Proc. Natl Acad. Sci. USA* **109**, 12040–12045 (2012).
40. Eichten, S. R. *et al.* Epigenetic and genetic influences on DNA methylation variation in maize populations. *Plant Cell* **25**, 2783–2797 (2013).
41. Regulski, M. *et al.* The maize methylome influences mRNA splice sites and reveals widespread paramutation-like switches guided by small RNA. *Genome Res.* **23**, 1651–1662 (2013).
42. Schmitz, R. J. *et al.* Epigenome-wide inheritance of cytosine methylation variants in a recombinant inbred population. *Genome Res.* **23**, 1663–1674 (2013).
43. Schmitz, R. J. *et al.* Patterns of population epigenomic diversity. *Nature* **495**, 193–198 (2013).
44. Li, Q. *et al.* Examining the causes and consequences of context-specific differential DNA methylation in maize. *Plant Physiol.* **168**, 1262–1274 (2015).
45. Kawakatsu, T. *et al.* Epigenomic diversity in a global collection of *Arabidopsis thaliana* accessions. *Cell* **166**, 492–505 (2016).
This study is an in-depth characterization of DNA methylation variation in >1,000 *A. thaliana* accessions.
46. Eichten, S. R., Stuart, T., Srivastava, A., Lister, R. & Borevitz, J. O. DNA methylation profiles of diverse *Brachypodium distachyon* align with underlying genetic diversity. *Genome Res.* **26**, 1520–1531 (2016).
47. Garg, R., Narayana Chevala, V., Shankar, R. & Jain, M. Divergent DNA methylation patterns associated with gene expression in rice cultivars with contrasting drought and salinity stress response. *Sci. Rep.* **5**, 14922 (2015).
48. Shen, X. *et al.* Natural CMT2 variation is associated with genome-wide methylation changes and temperature seasonality. *PLoS Genet.* **10**, e1004842 (2014).
49. Dubin, M. J. *et al.* DNA methylation in *Arabidopsis* has a genetic basis and shows evidence of local adaptation. *eLife* **4**, e05255 (2015).
50. Pignatta, D. *et al.* Natural epigenetic polymorphisms lead to intraspecific variation in *Arabidopsis* gene imprinting. *eLife* **3**, e03198 (2014).
51. Zhang, L. *et al.* A natural tandem array alleviates epigenetic repression of IPA1 and leads to superior yielding rice. *Nat. Commun.* **8**, 14789 (2017).
52. Deng, Y. *et al.* Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. *Science* **355**, 962–965 (2017).
53. Meng, D. *et al.* Limited contribution of DNA methylation variation to expression regulation in *Arabidopsis thaliana*. *PLoS Genet.* **12**, e1006141 (2016).
54. Cubas, P., Vincent, C. & Coen, E. An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* **401**, 157–161 (1999).
55. Manning, K. *et al.* A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat. Genet.* **38**, 948–952 (2006).
56. Chandler, V. L. Paramutation: from maize to mice. *Cell* **128**, 641–645 (2007).
57. Reinders, J. *et al.* Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Dev.* **23**, 939–950 (2009).
58. Johannes, F. *et al.* Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet.* **5**, e1000530 (2009).
References 57 and 58 describe the creation and phenotypic characterization of the first epiRIL populations.

59. Cortijo, S. *et al.* Mapping the epigenetic basis of complex traits. *Science* **343**, 1145–1148 (2014). **This article clearly documents the potential role of epigenetic variation in influencing quantitative traits in plants.**
60. Kooke, R. *et al.* Epigenetic basis of morphological variation and phenotypic plasticity in *Arabidopsis thaliana*. *Plant Cell* **27**, 337–348 (2015).
61. Zhang, Y. Y., Fischer, M., Colot, V. & Bossdorf, O. Epigenetic variation creates potential for evolution of plant phenotypic plasticity. *New Phytol.* **197**, 314–322 (2013).
62. Dapp, M. *et al.* Heterosis and inbreeding depression of epigenetic *Arabidopsis* hybrids. *Nat. Plants* **1**, 15092 (2015).
63. Hu, L. *et al.* Mutation of a major CG methylase in rice causes genome-wide hypomethylation, dysregulated genome expression, and seedling lethality. *Proc. Natl Acad. Sci. USA* **111**, 10642–10647 (2014).
64. Yamauchi, T., Johzuka-Hisatomi, Y., Terada, R., Nakamura, I. & Iida, S. The MET1b gene encoding a maintenance DNA methyltransferase is indispensable for normal development in rice. *Plant Mol. Biol.* **85**, 219–232 (2014).
65. Li, Q. *et al.* Genetic perturbation of the maize methylome. *Plant Cell* **26**, 4602–4616 (2014).
66. Richards, E. J. Inherited epigenetic variation — revisiting soft inheritance. *Nat. Rev. Genet.* **7**, 395–401 (2006).
67. Taudt, A., Colome-Tatche, M. & Johannes, F. Genetic sources of population epigenomic variation. *Nat. Rev. Genet.* **17**, 319–332 (2016).
68. Hollister, J. D. & Gaut, B. S. Epigenetic silencing of transposable elements: a trade-off between reduced transposition and deleterious effects on neighboring gene expression. *Genome Res.* **19**, 1419–1428 (2009).
69. Bender, J. & Fink, G. R. Epigenetic control of an endogenous gene family is revealed by a novel blue fluorescent mutant of *Arabidopsis*. *Cell* **83**, 725–734 (1995).
70. Gehring, M., Bubb, K. L. & Henikoff, S. Extensive demethylation of repetitive elements during seed development underlies gene imprinting. *Science* **324**, 1447–1451 (2009).
71. Hsieh, T. F. *et al.* Genome-wide demethylation of *Arabidopsis* endosperm. *Science* **324**, 1451–1454 (2009).
72. Zemach, A. *et al.* Local DNA hypomethylation activates genes in rice endosperm. *Proc. Natl Acad. Sci. USA* **107**, 18729–18734 (2010).
73. Slotkin, R. K. *et al.* Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* **136**, 461–472 (2009).
74. Ibarra, C. A. *et al.* Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. *Science* **337**, 1360–1364 (2012).
75. Park, K. *et al.* DNA demethylation is initiated in the central cells of *Arabidopsis* and rice. *Proc. Natl Acad. Sci. USA* **113**, 15138–15143 (2016).
76. Rodrigues, J. A. & Zilberman, D. Evolution and function of genomic imprinting in plants. *Genes Dev.* **29**, 2517–2531 (2015).
77. Yuan, J. *et al.* Both maternally and paternally imprinted genes regulate seed development in rice. *New Phytol.* http://dx.doi.org/10.1111/nph.14510 (2017).
78. Costa, L. M. *et al.* Maternal control of nutrient allocation in plant seeds by genomic imprinting. *Curr. Biol.* **22**, 160–165 (2012).
79. Calarco, J. P. *et al.* Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. *Cell* **151**, 194–205 (2012).
80. Hsieh, P. H. *et al. Arabidopsis* male sexual lineage exhibits more robust maintenance of CG methylation than somatic tissues. *Proc. Natl Acad. Sci. USA* **113**, 15132–15137 (2016).
81. Liu, R. *et al.* A DEMETER-like DNA demethylase governs tomato fruit ripening. *Proc. Natl Acad. Sci. USA* **112**, 10804–10809 (2015).
82. Satge, C. *et al.* Reprogramming of DNA methylation is critical for nodule development in Medicago truncatula. *Nat. Plants* **2**, 16166 (2016).
83. Kawakatsu, T. *et al.* Unique cell-type-specific patterns of DNA methylation in the root meristem. *Nat. Plants* **2**, 16058 (2016). **This dissection of cell type-specific methylation patterns in plants reveals mostly similar patterns, with the exception of columella cells.**
84. Pecinka, A. & Mittelsten Scheid, O. Stress-induced chromatin changes: a critical view on their heritability. *Plant Cell Physiol.* **53**, 801–808 (2012).
85. Secco, D. *et al.* Stress induced gene expression drives transient DNA methylation changes at adjacent repetitive elements. *eLife* **4**, e09343 (2015). **This detailed analysis of methylome and transcriptome response to phosphate stress finds evidence for expression-induced changes in methylation in response to abiotic stress in rice.**
86. Jiang, C. *et al.* Environmentally responsive genome-wide accumulation of *de novo Arabidopsis thaliana* mutations and epimutations. *Genome Res.* **24**, 1821–1829 (2014).
87. Wibowo, A. *et al.* 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 (2016).
88. Kaeppler, S. M., Kaeppler, H. F. & Rhee, Y. Epigenetic aspects of somaclonal variation in plants. *Plant Mol. Biol.* **43**, 179–188 (2000).
89. Peschke, V. M., Phillips, R. L. & Gengenbach, B. G. Discovery of transposable element activity among progeny of tissue culture — derived maize plants. *Science* **238**, 804–807 (1987).
90. Hirochika, H., Sugimoto, K., Otsuki, Y., Tsugawa, H. & Kanda, M. Retrotransposons of rice involved in mutations induced by tissue culture. *Proc. Natl Acad. Sci. USA* **93**, 7783–7788 (1996).
91. Rhee, Y., Sekhon, R. S., Chopra, S. & Kaeppler, S. Tissue culture-induced novel epialleles of a Myb transcription factor encoded by pericarp color1 in maize. *Genetics* **186**, 843–855 (2010).
92. Ong-Abdullah, M. *et al.* Loss of Karma transposon methylation underlies the mantled somaclonal variant of oil palm. *Nature* **525**, 533–537 (2015). **This study reports the identification of epialleles induced in tissue culture in clonally propagated oil palms.**
93. Tanurdzic, M. *et al.* Epigenomic consequences of immortalized plant cell suspension culture. *PLoS Biol.* **6**, 2880–2895 (2008).
94. Stroud, H. *et al.* Plants regenerated from tissue culture contain stable epigenome changes in rice. *eLife* **2**, e00354 (2013).
95. Stelpflug, S. C., Eichten, S. R., Hermanson, P. J., Springer, N. M. & Kaeppler, S. M. Consistent and heritable alterations of DNA methylation are induced by tissue culture in maize. *Genetics* **198**, 209–218 (2014).
96. Eichten, S. R. & Springer, N. M. Minimal evidence for consistent changes in maize DNA methylation patterns following environmental stress. *Front. Plant. Sci.* **6**, 308 (2015).
97. Song, Q. X. *et al.* Genome-wide analysis of DNA methylation in soybean. *Mol. Plant* **6**, 1961–1974 (2013).
98. Hagmann, J. *et al.* Century-scale methylome stability in a recently diverged *Arabidopsis thaliana* lineage. *PLoS Genet.* **11**, e1004920 (2015).
99. Le, T. N. *et al.* DNA demethylases target promoter transposable elements to positively regulate stress responsive genes in *Arabidopsis*. *Genome Biol.* **15**, 458 (2014).
100. Hollick, J. B. Paramutation and related phenomena in diverse species. *Nat. Rev. Genet.* **18**, 5–23 (2017).
101. Greaves, I. K. *et al.* Twenty-four-nucleotide siRNAs produce heritable *trans*-chromosomal methylation in F1 *Arabidopsis* hybrids. *Proc. Natl Acad. Sci. USA* **113**, E6895–E6902 (2016).
102. Greaves, I. K. *et al.* Trans chromosomal methylation in *Arabidopsis* hybrids. *Proc. Natl Acad. Sci. USA* **109**, 3570–3575 (2012).
103. Zhang, Q. *et al.* Methylation interactions in *Arabidopsis* hybrids require RNA-directed DNA methylation and are influenced by genetic variation. *Proc. Natl Acad. Sci. USA* **113**, E4248–E4256 (2016).
104. Jordan, W. T. & Schmitz, R. J. The shocking consequences of hybrid epigenomes. *Genome Biol.* **17**, 85 (2016).
105. Rigal, M. *et al.* Epigenome confrontation triggers immediate reprogramming of DNA methylation and transposon silencing in *Arabidopsis thaliana* F1 epihybrids. *Proc. Natl Acad. Sci. USA* **113**, E2083–E2092 (2016). **In this report, epihybrids between a wild-type parent and a hypomethylated parent reveal widespread redistribution of heterochromatin marks.**
106. Lei, M. *et al.* Regulatory link between DNA methylation and active demethylation in *Arabidopsis*. *Proc. Natl Acad. Sci. USA* **112**, 3553–3557 (2015).
107. Williams, B. P., Pignatta, D., Henikoff, S. & Gehring, M. Methylation-sensitive expression of a DNA demethylase gene serves as an epigenetic rheostat. *PLoS Genet.* **11**, e1005142 (2015).
108. Wendel, J. F., Jackson, S. A., Meyers, B. C. & Wing, R. A. Evolution of plant genome architecture. *Genome Biol.* **17**, 37 (2016).
109. Zhang, J. *et al.* Autotetraploid rice methylome analysis reveals methylation variation of transposable elements and their effects on gene expression. *Proc. Natl Acad. Sci. USA* **112**, E7022–E7029 (2015). **This study provides evidence for ploidy-induced changes in DNA methylation and gene expression in rice.**
110. Edgar, P. P. *et al.* Subgenome dominance in an interspecific hybrid, synthetic allopolyploid, and a 140 year old naturally established neo-allopolyploid monkeyflower. Preprint at *bioRxiv* https://doi.org/10.1101/094797 (2016).
111. Becker, C. *et al.* Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature* **480**, 245–249 (2011).
112. Schmitz, R. J. *et al.* Transgenerational epigenetic instability is a source of novel methylation variants. *Science* **334**, 369–373 (2011).
113. van der Graaf, A. *et al.* Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. *Proc. Natl Acad. Sci. USA* **112**, 6676–6681 (2015). **This study is a careful dissection of the rates of spontaneous change in DNA methylation in plants.**
114. Shaw, R. G., Byers, D. L. & Darmo, E. Spontaneous mutational effects on reproductive traits of *Arabidopsis thaliana*. *Genetics* **155**, 369–378 (2000).
115. Ossowski, S. *et al.* The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. *Science* **327**, 92–94 (2010).
116. Zheng, X. *et al.* Transgenerational epimutations induced by multi-generation drought imposition mediate rice plant's adaptation to drought condition. *Sci. Rep.* **7**, 39843 (2017).
117. Li, Q., Eichten, S. R., Hermanson, P. J. & Springer, N. M. Inheritance patterns and stability of DNA methylation variation in maize near-isogenic lines. *Genetics* **196**, 667–676 (2014).
118. Eichten, S. R. *et al.* Spreading of heterochromatin is limited to specific families of maize retrotransposons. *PLoS Genet.* **8**, e1003127 (2012).
119. Catoni, M. *et al.* DNA sequence properties that predict susceptibility to epiallelic switching. *EMBO J.* **36**, 617–628 (2017).
120. Lewsey, M. G. *et al.* Mobile small RNAs regulate genome-wide DNA methylation. *Proc. Natl Acad. Sci. USA* **113**, E801–E810 (2016).
121. Melnyk, C. W., Molnar, A. & Baulcombe, D. C. Intercellular and systemic movement of RNA silencing signals. *EMBO J.* **30**, 3553–3563 (2011).
122. Kasai, A., Bai, S., Hojo, H. & Harada, T. Epigenome editing of potato by grafting using transgenic tobacco as siRNA donor. *PLoS ONE* **11**, e0161729 (2016).
123. Jaligot, E. *et al.* Epigenetic imbalance and the floral developmental abnormality of the *in vitro*-regenerated oil palm *Elaeis guineensis*. *Ann. Bot.* **108**, 1453–1462 (2011).
124. Telias, A. *et al.* Apple skin patterning is associated with differential expression of MYB10. *BMC Plant Biol.* **11**, 93 (2011).
125. Xu, J., Tanino, K. K. & Robinson, S. J. Stable epigenetic variants selected from an induced hypomethylated *Fragaria vesca* population. *Front. Plant Sci.* **7**, 1768 (2016).
126. Amabile, A. *et al.* Inheritable silencing of endogenous genes by hit-and-run targeted epigenetic editing. *Cell* **167**, 219–232.e14 (2016).
127. Liu, X. S. *et al.* Editing DNA methylation in the mammalian genome. *Cell* **167**, 233–247.e17 (2016).
128. Park, M., Keung, A. J. & Khalil, A. S. The epigenome: the next substrate for engineering. *Genome Biol.* **17**, 183 (2016).
129. Bikard, D. *et al.* Programmable repression and activation of bacterial gene expression using an engineered CRISPR–Cas system. *Nucleic Acids Res.* **41**, 7429–7437 (2013).
130. Johnson, L. M. *et al.* SRA- and SET-domain-containing proteins link RNA polymerase V occupancy to DNA methylation. *Nature* **507**, 124–128 (2014).

In this study, the authors use zinc finger endonucleases to direct *de novo* DNA methylation in *A. thaliana*.
131. Griffin, P. T., Niederhuth, C. E. & Schmitz, R. J. A. Comparative analysis of 5-azacytidine- and zebularine-induced DNA demethylation. *G3 (Bethesda)* **6**, 2773–2780 (2016).
132. Waddington, C. H. Canalization of development and genetic assimilation of acquired characters. *Nature* **183**, 1654–1655 (1959).
133. Wolff, P. *et al.* High-resolution analysis of parent-of-origin allelic expression in the *Arabidopsis* endosperm. *PLoS Genet.* **7**, e1002126 (2011).
134. Zhang, M. *et al.* Genome-wide high resolution parental-specific DNA and histone methylation maps uncover patterns of imprinting regulation in maize. *Genome Res.* **24**, 167–176 (2014).
135. Moreno-Romero, J., Jiang, H., Santos-Gonzalez, J. & Kohler, C. Parental epigenetic asymmetry of PRC2-mediated histone modifications in the *Arabidopsis* endosperm. *EMBO J.* **35**, 1298–1311 (2016).
136. Probst, A. V. & Mittelsten Scheid, O. Stress-induced structural changes in plant chromatin. *Curr. Opin. Plant Biol.* **27**, 8–16 (2015).
137. Chen, X., Liu, X., Zhao, Y. & Zhou, D. X. Histone H3K4me3 and H3K27me3 regulatory genes control stable transmission of an epimutation in rice. *Sci. Rep.* **5**, 13251 (2015).
138. Guo, Z. *et al.* Global epigenomic analysis indicates that epialleles contribute to allele-specific expression via allele-specific histone modifications in hybrid rice. *BMC Genomics* **16**, 232 (2015).
139. Song, X. J. *et al.* Rare allele of a previously unidentified histone H4 acetyltransferase enhances grain weight, yield, and plant biomass in rice. *Proc. Natl Acad. Sci. USA* **112**, 76–81 (2015).
140. Jaskiewicz, M., Conrath, U. & Peterhansel, C. Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Rep.* **12**, 50–55 (2011).
141. Ding, Y., Fromm, M. & Avramova, Z. Multiple exposures to drought 'train' transcriptional responses in *Arabidopsis*. *Nat. Commun.* **3**, 740 (2012).
142. Sani, E., Herzyk, P., Perrella, G., Colot, V. & Amtmann, A. Hyperosmotic priming of *Arabidopsis* seedlings establishes a long-term somatic memory accompanied by specific changes of the epigenome. *Genome Biol.* **14**, R59 (2013).
143. Du, J. *et al.* Dual binding of chromomethylase domains to H3K9me2-containing nucleosomes directs DNA methylation in plants. *Cell* **151**, 167–180 (2012).
144. Deleris, A. *et al.* Loss of the DNA methyltransferase MET1 Induces H3K9 hypermethylation at PcG target genes and redistribution of H3K27 trimethylation to transposons in *Arabidopsis thaliana*. *PLoS Genet.* **8**, e1003062 (2012).
This study shows how loss of DNA methylation at some loci leads to transcriptional repression by the Polycomb complex.
145. Greenberg, M. V. *et al.* Interplay between active chromatin marks and RNA-directed DNA methylation in *Arabidopsis thaliana*. *PLoS Genet.* **9**, e1003946 (2013).
146. Laprell, F., Finkl, K. & Muller, J. Propagation of Polycomb-repressed chromatin requires sequence-specific recruitment to DNA. *Science* **356**, 85–88 (2017).
147. Wang, X. & Moazed, D. DNA sequence-dependent epigenetic inheritance of gene silencing and histone H3K9 methylation. *Science* **356**, 88–91 (2017).
148. Zhang, L. *et al.* Identification and characterization of an epi-allele of FIE1 reveals a regulatory linkage between two epigenetic marks in rice. *Plant Cell* **24**, 4407–4421 (2012).
149. Zabet, N. R., Catoni, M., Prischi, F. & Paszkowski, J. Cytosine methylation at CpCpG sites triggers accumulation of non-CpG methylation in gene bodies. *Nucleic Acids Res.* **45**, 3777–3784 (2017).
150. Du, J., Johnson, L. M., Jacobsen, S. E. & Patel, D. J. DNA methylation pathways and their crosstalk with histone methylation. *Nat. Rev. Mol. Cell Biol.* **16**, 519–532 (2015).
151. Bewick, A. J. *et al.* The evolution of CHROMOMETHYLASES and gene body DNA methylation in plants. *Genome Biol.* **18**, 65 (2017).
152. West, P. T. *et al.* Genomic distribution of H3K9me2 and DNA methylation in a maize genome. *PLoS ONE* **9**, e105267 (2014).

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