Chapter 3
The Role of Germinally Inherited Epialleles in Plant Breeding: An Update

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Abstract Plant breeding focuses on repeated selection of individuals with desired traits from phenotypically variable populations. Breeders may be able to explain the broad sense heritability for a trait, the proportion of the total trait variance between genetically distinct lines compared to within a line, or the narrow sense heritability, the proportion of the trait variation that is due to the additive effects of genes. However, breeders rarely know the underlying causes of the observed genetic variation. In this chapter, we take a trait-focused approach to review the degree to which plant variation is due to epigenetic variation and to what degree epigenetic factors, mainly DNA methylation, are suitable for selection in plant breeding. This chapter is an update from a chapter published 4 years ago that highlighted that pure epigenetic variation (epigenetic differences across individuals not caused by DNA differences) is rare and often unstable and thus plays a small role in plant improvement. Our thesis has remained unchanged, but we supplement this text with additional examples, and we highlight those rare situations where pure alleles or facilitated epigenetic alleles (alleles that are caused by a DNA polymorphism but are maintained independently of that polymorphism) may be beneficial to plant improvement.

3.1 Introduction

The term 'epigenetics' has a number of definitions. Waddington (1942) used the term to explain how one genome gives rise to multiple cell lineages that follow diverse developmental trajectories (Waddington 1942). In other words, epigenetics referred to mechanisms that enable the developmentally appropriate expression of genes. In Waddington's conception, epigenetic information laid down in

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development is erased during gametogenesis, consistent with the Mendelian principle that genes passed across generations are unaltered by developmental or environmental stimuli. More recently, pure epigenetics has been described as meiotically heritable changes in gene function that are not due to differences in nucleotide sequence. Following this definition, an organism can impose chemical changes to DNA or chromatin within a germ cell, and these changes can be transmitted to the subsequent generation. Epimutation is a process that generates an epiallele, and the term epiallele refers to a gene with distinct biochemical modifications. Thus, a trait that both varies within a population because of polymorphic nucleotide sequence(s) and is correlated between parent/offspring pairs because of shared nucleotide sequence(s) exhibits genetic inheritance. A trait that both varies within a population because of variable chromatin structures and is correlated between parent/offspring pairs because of these structures exhibits epigenetic inheritance.

In this chapter, we first review how the inheritance of variable chromatin states (mainly variation in DNA methylation)—induced spontaneously, chemically, or genetically—can contribute to phenotypic variation. We then address the stability of epialleles across generations and the frequency at which stable epialleles occur in plant genomes. Finally, we highlight the role of epigenetic variation in plant breeding.

3.2 Changes in DNA Methylation Can Be Pure, Facilitated, or Genetically Dependent

Most epialleles characterized to date are marked by changes in the status of DNA methylation. These epigenetic modifications are easy to identify and the processes by which they are directed, maintained, and removed are well understood, making them attractive to investigate. Before examining specific instances of methylation-based epialleles and how they can be exploited in plant breeding, it is important to understand the relationship between cytosine methylation and the primary sequence of DNA. While pure epialleles do occur (i.e. those changes in DNA methylation that are purely epigenetic and have no dependence on DNA sequence), there are also epialleles that are either partially (facilitated epialleles) or fully (obligate epialleles) dependent on DNA polymorphisms and thus have a genetic dependence (Richards 2006). A solid example of a facilitated epiallele has been described for the *FOLT1* gene (Durand et al. 2012). *FOLT1* is a folate transporter whose expression can be silenced via increases in cytosine methylation. These changes in methylation are actually directed by truncated copies of a second, but related gene, *FOLT2*, that are located on a different chromosome. siRNAs originating from these truncated copies direct the hypermethylation at *FOLT1* and the subsequent silencing. If *FOLT1* silencing was completely dependent on the presence of the truncated copies of *FOLT2*, then this example would describe an obligate epiallele; however, silencing of *FOLT1* remains after the siRNA-derived loci are segregated away, indicating that

specific loci are necessary for initiation of the epiallele but are not required for its maintenance through generations. An example of an obligate epiallele has been elucidated by Woo et al. (2007). In this case, demethylation of centromeric repeats was identified in an Arabidopsis accession carrying a mutation in *VARIANT IN METHYLATION 1*. Occurring only in the presence of vim1, this epiallele is of the obligate category and is completely dependent on DNA polymorphism.

Facilitated and obligate epigenetic polymorphisms show greater stability than pure epialleles and are thus likely to be the most useful in plant breeding efforts geared towards generation of highly stable and heritable traits. Here, we describe a variety of pure and facilitated epialleles and bring attention to those types that are likely candidates for integration into crop improvement programs.

3.3 Meiotically Inherited Epigenetic Differences Can Cause Phenotypic Variation

Meiotically heritable epigenetic modifications are of interest to plant breeders because they can direct changes in phenotype that, in some cases, provide stable improvements to plant phenotype. In this chapter we focus on cytosine methylation (also commonly referred to as DNA methylation). The molecular processes underlying cytosine methylation are thoroughly described. The manipulation of DNA methylation via chemical treatment and through the use of genetic mutants is also well studied. While the examples of the positive relationships between changes in DNA methylation and changes in plant phenotype are numerous, it is important to understand how pure and facilitated epigenetic alleles are created and their transgenerational stability before determining their relevance to plant breeding. Here, we describe a variety of instances where changes in DNA methylation that have occurred spontaneously, following chemical treatment, at genes paired with certain alleles in heterozygotes, or in genomes with defective DNA methylation machinery, have resulted in changes to plant phenotype.

Some remarkable examples of pure epigenetic modifications have been found to occur spontaneously in nature (Table 3.1). One of the best known examples comes from a toadflax (*Linaria vulgaris*) mutant, originally described by Linnaeus, which has radially symmetric flowers rather than the wild-type bilaterally symmetric flowers (Gustafsson 1979). Cubas et al. (1999) mapped the floral shape difference to a *cycloidea* type gene (*Lcyc*). The mutant and wild-type alleles differ at a single nucleotide that does not explain the phenotypic difference (Cubas et al. 1999), but instead chromatin state seems to be the key factor distinguishing wild-type and mutant alleles. Among an F2 population derived from a cross of wild-type and mutant plants, the radially symmetric floral trait correlates perfectly with the cytosine methylation status of Sau3A restriction enzyme recognition sites (Cubas et al. 1999). In other words, plants with radially symmetrical flowers have high cytosine methylation upstream and within the coding sequences of *Lcyc*. In tomato, one

Table 3.1 Examples of epialleles described in this chapter

Species	Locus	Nature of change	Trait affected	Refs
L. vulgaris	*Lcyc*	Spontaneous	Floral architecture	Gustafsson (1979), Cubas et al. (1999)
Tomato	*Cnr*	Spontaneous	Skin pigmentation and fruit ripening	Thompson et al. (1999), Manning et al. (2006)
Rice	*QQS*	Spontaneous	Starch metabolism	Silveira et al. (2013)
Rice	*Epi-d1*	Spontaneous	Height	Miura et al. (2009)
Zea mays	*B1*	Spontaneous (paramutation)	Pigmentation	Coe (1966), Patterson et al. (1993, 1995), Stam et al. (2002)
Flax	?	Induced (5azaC)	Height, flowering time, and leaf number	Fieldes (1994), Fieldes et al. (2005)
Rice	?	Induced (azadC)	Height and pathogen resistance	Akimoto et al. (2007)
Triticale	?	Induced (5azaC)	Height, tillering, and flowering time	Heslop-Harrison (1990)
Strawberry	?	Induced (5azaC)	Flowering time and rosette diameter	Xu et al. (2016a, b)
Maize	*Spm*	Spontaneous	Anthocyanin production	McClintock (1958, 1965), Peterson (1966), Fowler and Peterson (1978), Banks et al. (1988), Fedoroff (1999)
Arabidopsis	*SUP*	Mutagen	Floral morphology	Jacobsen and Meyerowitz (1997), Ito et al. (2003)
Arabidopsis	*FWA*	Mutagen	Flowering time	Soppe et al. (2000)

dominant locus, *colourless non-ripening* (*Cnr*), causes plants to generate fruit with a colourless pericarp, inhibited softening, and reduced ethylene production (Thompson et al. 1999). The mutation was mapped to a 95 kb interval, but the nucleotide sequences of mutant and wild-type alleles were identical (Manning et al. 2006). An open reading frame with reduced expression in the mutant fruit compared to the wild-type fruit was identified as a *SQUAMOSA promoter binding-like* gene (*SPL*) transcription factor. A 286-bp region located 2.4 kb upstream of the gene is hypermethylated in mutant plants relative to the wild type (Manning et al. 2006). Similarly, plants homozygous for *clark kent* (*clk*) alleles of the *A. thaliana* *SUPERMAN* gene have a higher number of stamens and carpels than do wild-type plants (Bowman et al. 1992). *clk* and wild-type alleles have no sequence polymorphisms but the *clk* allele is extensively methylated relative to the wild-type allele (Jacobsen and Meyerowitz 1997). More recently an epiallele of the *QSS* (*Qua-Quine Starch*) gene that is involved in starch metabolism in Arabidopsis has been identified (Silveira et al. 2013). This epiallele appears spontaneously in nature and is explained by methylation of repeat elements in the 5′ region of the gene (Table 3.1) (Silveira et al. 2013).

Treatment of plants with DNA methylation inhibitors, such as 5-azacytidine (5azaC) and 5-azadeoxycytidine (azadC), can induce pure epigenetic, heritable, phenotypic changes, though it is not always known if the resulting phenotypic changes are truly pure, facilitated, or because the inhibitors may be mutagenic (Fig. 3.1). For example, Fieldes (1994) induced heritable phenotypic changes in flax using a 5azaC treatment. Relative to untreated plants, the plants growing from treated seeds were often shorter, had fewer leaves on the main stem, and had reduced flowering times (Table 3.1) (Fieldes 1994). From first generation progeny of treated
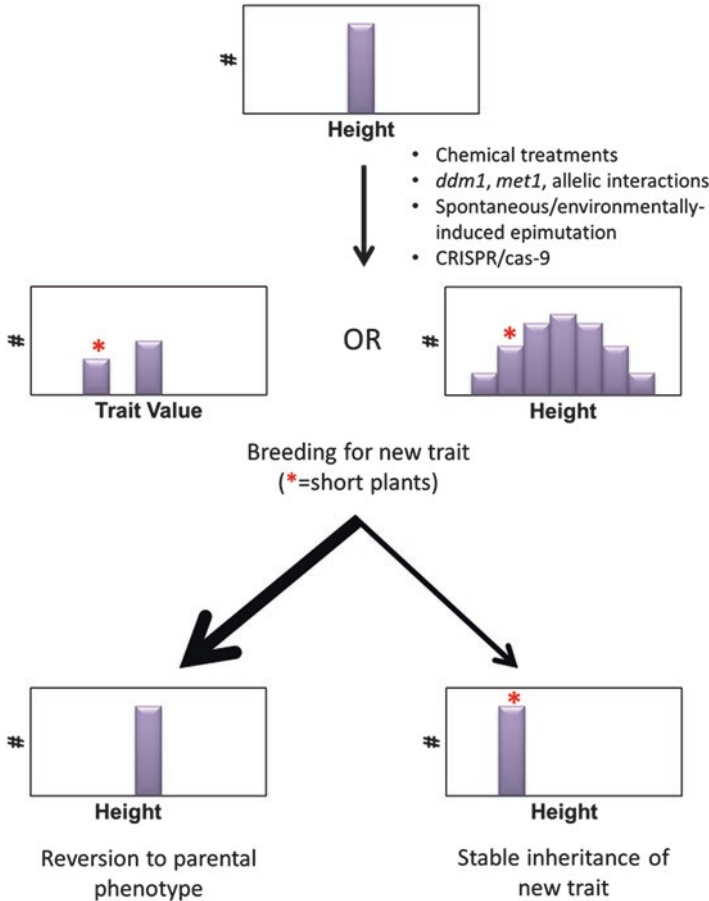

Fig. 3.1 Epimutations induced by several phenomena can generate phenotypic novelty that is in some cases stably inherited. Within the histograms above, the X axis represents a trait value for a plant, for example, plant height. The Y axis represents the number of individuals within a population with that trait value. New discrete or continuous trait values arise because of epimutation. The *asterisk* represents a new, favourable trait value. The *arrows* represent the relative frequency of outcomes of selection for the *asterisk* plants. On the *left*, selection was not successful. The trait has reverted to its ancestral value. On the *right*, selection successfully shifted the trait value of the population

plants, Fieldes selected six lines of flax that were short and early flowering (Fieldes 1994). Flax is self-pollinating, and these traits were stably transmitted to the next generation and many generations following that (Amyot 1997; House 2010). Additionally, flowering time variation in populations derived from crossing the early flowering line to the wild type indicated that at least three independent epialleles contribute to early flowering (Fieldes and Amyot 1999). In Triticale, a wheat × rye hybrid plant, plants grown from seeds treated with 5azaC have a number of heritable, phenotypic differences relative to plants from untreated seeds (Table 3.1) (Heslop-Harrison 1990). Specifically, one 5azaC treatment resulted in plants that were taller than controls, had increased tillering and an increased time to maturity, and these novel traits persisted through two subsequent generations, at which point the study concluded (Heslop-Harrison 1990). Akimoto et al. (2007) noted that two plants grown from a population of 100 rice seeds (*Oryza sativa* spp. *japonica*, 'Yamada-nishiki') treated with the DNA demethylating agent 5-azadeoxycytidine (azadC) differed from plants grown from untreated seeds. Most remarkable was a line that was dwarf and flowered 10–14 days early (Table 3.1). Similarly, chemically induced heritable variation has been observed in other crops, including Brassica (altered leaf morphology, reduced number of anthers, altered phyllotaxy, deformed flowers, and change in the time to flowering) (King 1995), rice (dwarfism and delayed ear emergence) (Sano et al. 1990), and Melandrium (appearance of bisexual flowers on a normally dioecious plant) (Janoušek et al. 1996). Recently, 5-azaC was used to induce changes in cytosine methylation in strawberry (Xu et al. 2016a) and through repeated selection, stable early flowering lines were established (Table 3.1) (Xu et al. 2016b). These strawberry studies are of significance because they provide evidence that changes in cytosine methylation can be induced by chemical treatment, can alter important traits, and be selected via artificial selection.

Mutations within genes important for maintaining DNA methylation also act as epimutagens and generate facilitated, heritable epialleles. For example, the *Arabidopsis thaliana* gene *DDM1* (*Deficient in DNA Methylation 1*) encodes an ATPase chromatin remodeler that is involved in the maintenance of DNA methylation in both CG and non-CG sequence contexts (Jeddeloh et al. 1999) and in the silencing of repeat elements such as transposons (Hirochika et al. 2000; Singer et al. 2001; Miura et al. 2001). Genomic DNA of the *ddm1* mutant is hypomethylated throughout the genome (Vongs et al. 1993). *ddm1* plants have weak phenotypes, but after several generations of selfing, novel traits related to leaf structure, flowering time, flower structure, both increased and decreased apical dominance, and reduced internode length arise at high frequency (Kakutani et al. 1996). Some epialleles that appear within the *ddm1* mutant background, such as that determining the *bns* phenotype, are stably inherited (Kakutani et al. 1996; Soppe et al. 2000; Saze and Kakutani 2007). METHYLTRANSFERASE1 (MET1) is also required for propagating CG methylation during DNA replication, and *Arabidopsis* (ecotype C24) *MET1* antisense lines show the heritable effects of aberrant DNA methylation patterns through the gradual loss of CG methylation (Finnegan et al. 1996). A number of traits arise in *met1* lines including reduced apical dominance, altered flowering

time, altered floral morphology, decreased plant size, and altered leaf shape and size (Finnegan et al. 1996). As with *ddm1* mutants, floral traits persist in individuals without the silencing alleles (Finnegan et al. 1996).

Some trait variation is also caused by allelic interactions between homologous alleles, which is also referred to as paramutation, a type of facilitated epiallele. While it is clear that there are genetic requirements for paramutation (Springer and McGinnis 2015), the complete mechanism underlying the process has yet to be determined. Studies of maize pigmentation inheritance have revealed a number of these scenarios. Brink (1956), who was studying anthocyanin biosynthesis in maize, noted the changing effects of alleles across generations. Specifically, he found that the effect of the *R-r* allele, which typically confers full pigmentation in seeds, varied in its effect on seed colour depending on an association with another allele, *R-st*, an allele which results in stippled pigmentation. If inherited with *R-st*, the resulting *R-st*/*R-r* progeny had lower-than-expected levels of pigmentation. When *R-st*/*R-r* was crossed with *r/r* individuals, the progeny carrying the *R-r* allele also had much reduced pigmentation compared to the expectation of fully pigmented seeds. In fact what Brink was observing was the paramutagenic effect of *R-st* on *R-r*. The paramutagenic effect was transferred to *R-r* and remained for several generations but did eventually revert after repeatedly being inherited in the absence of *R-st*. The *booster1* (*b1*) locus in maize (Coe 1966) also regulates the production of anthocyanin pigments. Plants homozygous for the *B-I* (*B-Intense*) allele at the *b1* gene have dark purple pigmentation and high levels of gene expression, whereas plants homozygous for the *B'* allele are lightly pigmented (Coe 1966) and have low levels of transcription at the *b1* gene (10–20-fold lower than *B-I* homozygotes) (Patterson et al. 1993). In heterozygotes that carry both the *B-I* allele and the *B'* allele, *B-I* is converted (paramutated) to *B'* with 100% frequency (Coe 1966). The new *B'* allele is designated *B'*, and is able to paramutate a *B-I* allele to *B'* in the following generation (Coe 1966). A region of tandem repeats ~6 kb in length and ~100 kb upstream of the *b1* gene is crucial for the paramutagenicity and the paramutability of the *B'* and *B-I* alleles (Stam et al. 2002). RNA-dependent RNA polymerase, *mediator of paramutation1* (*mop1*) is necessary for paramutation to occur (Alleman et al. 2006), indicating that double stranded RNA is very likely a key factor that changes paramutable alleles to paramutagenic alleles, though this research is ongoing (Springer and McGinnis 2015). Paramutation likely describes a very small percentage of epialleles (Eichten et al. 2013).

3.4 The Stability of Facilitated and Pure Epialleles Across Generations

Epialleles may, in certain circumstances, have two attributes that suggest utility in plant breeding. These useful epialleles have beneficial effects on traits, and these effects are heritable. Many epialleles are also remarkably stable across generations.

Fieldes et al. (2005) demonstrated that seed from self-pollinated, early flowering flax lines generated by 5azaC treatment did not revert and continued to flower early for over eight generations. The level of total genomic cytosine methylation within early flowering plants was also stably inherited (Fieldes et al. 2005). Fieldes et al. estimated that 5–8% of cytosines were methylated in the early flowering lines, while 14% of cytosines were methylated within the control lines (14%) (Fieldes et al. 2005). Akimoto et al. (2007) reported that the dwarf trait generated by azadC treatment in rice was stably inherited over nine generations. The same line had higher resistance to infection by a *Xanthomonas oryzae* strain than did the wild-type line (Akimoto et al. 2007). Although in these instances it has not been established if the cause of the phenotype of interest is purely epigenetic, these examples provide evidence for the stability of traits induced by treatment with DNA hypomethylating agents. The *Cnr* pure epiallele described above also has high stability. Between the years of 1993 and 2006, more than 3000 mutant plants with the colourless phenotype were grown, and of those plants a revertant 'ripening sector' containing wild-type pigmentation was observed on only three fruits on three separate plants (Manning et al. 2006). The *B′* epiallele in maize is also extremely stable once formed (Coe 1966; Stam et al. 2002). Patterson et al. reported scoring over 20,000 progeny of *B′/B′* plants and seeing no revertants to *B-I* (Coe 1966; Patterson et al. 1993).

Despite the examples of persistent and pure epialleles, stable inheritance of traits caused by pure epialleles seems to be the exception rather than the rule. Among the epialleles generated by chemical treatment that have phenotypic effects, many lose their effect over generations and only in specific and rare instances remain stable over many generations (Fieldes 1994). In maize, *R′* (the paramutated, and temporarily paramutable version of *R-r*) can readily revert to *R-r* (Brink 1956). The anthocyanin traits conditional on *Spm* activity, as described by McClintock, are reversible and highly changeable. For instance, she observed that elements can remain silent for multiple generations after which they return spontaneously, and at a low frequency, to an active state (McClintock 1958, 1965; Fedoroff 1999). The radially symmetrical form of *L. vulgaris* is widespread (Gustafsson 1979). However, from a segregating population derived from intermating five F1 individuals from a cross between a radially flowered mutant and bilaterally flowered wild-type plant, only 5 of 39 plants (13%) have radially symmetric flowers (Cubas et al. 1999). In addition, among the five plants with radially symmetrical flowers, four had partial reversions to the wild-type phenotype (Cubas et al. 1999). In rice, a metastable epiallele called *Epi-d1* has been identified (Miura et al. 2009) that confers silencing of the *DWARF1* gene and causes a short stature phenotype. Typical of pure alleles, *Epi-d1* plants are often chimeric, showing a combination of dwarf and wild-type tillers (Miura et al. 2009).

Two experiments in *Arabidopsis thaliana* suggest traits due to *ddm1-* and *met1-* induced epigenetic variation can be stably inherited through many generations. Reinders et al. (2009) generated RILs derived from a cross between a wild-type plant and homozygous *met1-3* mutant. Plants homozygous for the wild-type *met1* allele in the F2 were selected and these genotypes were inbred for six generations. Reinders et al. (2009) reported that induced changes in flowering time, plant growth

(biomass), and salt stress tolerances appeared stable in particular epi-RILs. Similarly, Johannes et al. (2009) studied the effects of inherited hypomethylated epialleles created in a *ddm1* mutant background on plant height and flowering time variation. Johannes et al. did not estimate narrow sense heritability, but genetic differences among RIL lineages are surprisingly high for flowering time ($H^2 = 0.26$) and plant height ($H^2 = 0.32$). The trait variance explained among the RILs is about 1/3 to 1/10 the variance explained across a diverse set of natural accessions (Roux et al. 2011). These experiments are designed such that variation among the RILs should be attributed to facilitated or pure epigenetic differences. Nonetheless, some trait variation may be genetic, although almost certainly a small proportion. Parental lines, despite having a recent, shared common ancestor, likely have some polymorphic DNA sites. Mutations could also occur during inbreeding (Ossowski et al. 2010).

3.5 Obligate Epialleles Greatly Outnumber Facilitated and Pure Alleles Within Plant Genomes

While pure epialleles that have no relationship with genetic variation are remarkable and of interest, the reality is that many epialleles are associated with genetic variants. In Arabidopsis, the data ranges from a relatively small percentage (18%) of epialleles being associated with DNA sequence polymorphisms (Dubin et al. 2015), to a moderate level of association (35%) (Schmitz et al. 2013b), to a high level of association (~50%) (Hagmann et al. 2015). Interestingly, even the higher estimates for Arabidopsis are low in comparison to those in some field crops. Strong evidence for the imbalance in the frequency of pure epialleles to those with at least partial genetic dependence has been found in soybean. An analysis of 83 RIL and parent methylomes revealed that 91% of DMRs (differentially methylated regions) were associated with genetic differences between RILs, and the remaining 9% of DMRs possibly represent a combination of pure and facilitated epialleles (Schmitz et al. 2013a). Eichten et al. (2013) investigated DMRs in maize cultivars and found evidence for potentially pure epialleles, but also found evidence for regions that are differentially methylated and dependent on associated differences in genotype. More specifically, 51% of DMRs examined were associated with local SNPs (Eichten et al. 2013). The remaining DMRs may represent pure epialleles, but more likely are a combination of pure epialleles, alleles with trans-acting regulatory factors, and alleles for which there are not any SNPs in linkage disequilibrium (Eichten et al. 2013). In another study that examined the methylomes of maize genotypes B73, Mo17, and 9 RILs from a B73 × Mo17 cross, researchers determined that most of the CG methylation segregated with the parental genotype, indicating that those epivariants were associated with genetic variants (Regulski et al. 2013). Genomes with numerous repetitive elements, such as many crop genomes, may have a higher chance of generating new, beneficial epialleles than compact genomes with few such elements.

3.6 Conclusion: The Importance of Epigenetics for Past and Future Crop Breeding

Judging from published research, the molecular basis of most heritable trait variation within and between breeding populations is overwhelmingly genetic. This fact suggests to us that past plant breeding has likely minimally utilized pure epigenetic variation. Researchers may have *a priori* examined traits that were more likely under genetic than epigenetic control. For example, chromatin variation may be a more common regulatory mechanism for genes with limited or low activity (Gemma et al. 2013). Researchers may have also not reported cases in which trait variation cannot be attributed to a DNA polymorphism. Although such scenarios are possible, they unlikely explain the predominance of DNA polymorphisms as causative factors. Instead, many genes may have chromatin structures that have evolved to be resistant to epimutation. Given the importance of chromatin structure regulation throughout development, a mutation that causes stable inheritance of an epigenetic state on an allele may well be deleterious (Jorgensen 1993).

Nonetheless, plant breeding requires significant traits to vary and for selection on those traits to be effective. As noted above, facilitated and pure epigenetic differences can cause meaningful trait variation that is heritable. In addition, novel variation is generated far more quickly from single residue epimutation than from DNA mutations, and larger, stable differentially methylated regions arise at the same rate as genetic mutations (Becker et al. 2011). Thus, although pure and facilitated epimutations are rare and often unstable, these epialleles may be promising sources of new trait variation. Novel epialleles would especially play a role in breeding populations where there is little genetic variation. The approach taken for generating novel epialleles within a plant population is dependent on the species, with genome size and propagation method being factors to consider. For example, while methods, such as epiRIL development, have been successful in plants with simple genomes, such as Arabidopsis, crop plants with larger and more complex genomes, such as maize (Li et al. 2014) and rice (Hu et al. 2014), have been recalcitrant to this method. Chemical treatments provide an efficient means for discovering effects of hypomethylation since there are no specific targets of the demethylation and the effects can be widespread. Such treatments have been used to effectively alter important traits in crop species (Sano et al. 1990; Heslop-Harrison 1990; Fieldes 1994; King 1995; Janoušek et al. 1996; Akimoto et al. 2007; Xu et al. 2016a, b), but it is possible that the underlying cause of the altered phenotype may still, in part or entirely, be due to genetic differences. More recent developments in technology are bringing forward methods for directing site-specific changes in methylation status that might provide a means for inducing stable changes in methylation within genes of interest. For example, the CRISPR-cas9 system has been used to increase methylation in a site-specific manner in mammalian cells that resulted in facilitated epigenetic silencing of target genes (Vojta et al. 2016). This technology will not only provide a means for directing specific increases and decreases in methylation that have already

proven to drive meaningful phenotypic changes, but will also provide a means for studying the direct effects of novel changes in methylation rather than relying solely on correlations between changes in methylation and changes in gene expression.

References

Akimoto K, Katakami H, Kim H-J et al (2007) Epigenetic inheritance in rice plants. Ann Bot 100:205–217. https://doi.org/10.1093/aob/mcm110

Alleman M, Sidorenko L, McGinnis K et al (2006) An RNA-dependent RNA polymerase is required for paramutation in maize. Nature 442:295–298. https://doi.org/10.1038/nature04884

Amyot LM (1997) Characterization of 5-azacytidine-induced early flowering lines in flax. University of Waterloo

Banks JA, Masson P, Fedoroff N (1988) Molecular mechanisms in the developmental regulation of the maize Suppressor-mutator transposable element. Genes Dev 2:1364–1380. https://doi.org/10.1101/gad.2.11.1364

Becker C, Hagmann J, Müller J et al (2011) Spontaneous epigenetic variation in the Arabidopsis thaliana methylome. Nature 480:245–249. https://doi.org/10.1038/nature10555

Bowman JL, Sakai H, Jack T et al (1992) SUPERMAN, a regulator of floral homeotic genes in Arabidopsis. Development 114:599–615

Brink RA (1956) A genetic change associated with the R Locus in maize which is directed and potentially reversible. Genetics 41:872–889

Coe EH (1966) The properties, origin, and mechanism of conversion-type inheritance at the b locus in maize. Genetics 53:1035–1063

Cubas P, Vincent C, Coen E (1999) An epigenetic mutation responsible for natural variation in floral symmetry. Nature 401:157–161. https://doi.org/10.1038/43657

Dubin MJ, Zhang P, Meng D et al (2015) DNA methylation in Arabidopsis has a genetic basis and shows evidence of local adaptation. eLife 4:e05255. https://doi.org/10.7554/eLife.05255

Durand S, Bouché N, Perez Strand E et al (2012) Rapid establishment of genetic incompatibility through natural epigenetic variation. Curr Biol 22:326–331. https://doi.org/10.1016/j.cub.2011.12.054

Eichten SR, Briskine R, Song J et al (2013) Epigenetic and genetic influences on DNA methylation variation in maize populations. Plant Cell 25:2783–2797. https://doi.org/10.1105/tpc.113.114793

Fedoroff NV (1999) The Suppressor-mutator element and the evolutionary riddle of transposons. Genes Cells 4:11–19. https://doi.org/10.1046/j.1365-2443.1999.00233.x

Fieldes MA (1994) Heritable effects of 5-azacytidine treatments on the growth and development of flax (Linum usitatissimum) genotrophs and genotypes. Genome 37:1–11. https://doi.org/10.1139/g94-001

Fieldes MA, Amyot LM (1999) Epigenetic control of early flowering in flax lines induced by 5-azacytidine applied to germinating seed. J Hered 90:199–206. https://doi.org/10.1093/jhered/90.1.199

Fieldes MA, Schaeffer SM, Krech MJ, Brown JCL (2005) DNA hypomethylation in 5-azacytidine-induced early-flowering lines of flax. Theor Appl Genet 111:136–149. https://doi.org/10.1007/s00122-005-2005-9

Finnegan EJ, Peacock WJ, Dennis ES (1996) Reduced DNA methylation in Arabidopsis thaliana results in abnormal plant development. Proc Natl Acad Sci U S A 93:8449–8454

Fowler RG, Peterson PA (1978) An altered state of a specific en regulatory element induced in a maize tiller. Genetics 90:761–782

Gemma C, Ramagopalan SV, Down TA et al (2013) Inactive or moderately active human promoters are enriched for inter-individual epialleles. Genome Biol 14:1–11. https://doi.org/10.1186/gb-2013-14-5-r43

Gustafsson Å (1979) Linnaeus' Peloria: the history of a monster. Theor Appl Genet 54:241–248. https://doi.org/10.1007/BF00281206

Hagmann J, Becker C, Müller J et al (2015) Century-scale methylome stability in a recently diverged arabidopsis thaliana lineage. PLOS Genet 11:e1004920. https://doi.org/10.1371/journal.pgen.1004920

Heslop-Harrison JS (1990) Gene expression and parental dominance in hybrid plants. Development 108:21–28

Hirochika H, Okamoto H, Kakutani T (2000) Silencing of retrotransposons in arabidopsis and reactivation by the ddm1 mutation. Plant Cell Online 12:357–368. https://doi.org/10.1105/tpc.12.3.357

House M (2010) Molecular studies of 5-azacytidine-induced early-flowering lines of flax. Theses Dissertation Compr

Hu L, Li N, Xu C et al (2014) Mutation of a major CG methylase in rice causes genome-wide hypomethylation, dysregulated genome expression, and seedling lethality. Proc Natl Acad Sci 111:10642–10647. https://doi.org/10.1073/pnas.1410761111

Ito T, Sakai H, Meyerowitz EM (2003) Whorl-specific expression of the superman gene of arabidopsis is mediated by cis elements in the transcribed region. Curr Biol 13:1524–1530. https://doi.org/10.1016/S0960-9822(03)00612-2

Jacobsen SE, Meyerowitz EM (1997) Hypermethylated superman epigenetic alleles in arabidopsis. Science 277:1100–1103. https://doi.org/10.1126/science.277.5329.1100

Janoušek B, Široký J, Vyskot B (1996) Epigenetic control of sexual phenotype in a dioecious plant, Melandrium album. Mol Gen Genet 250:483–490. https://doi.org/10.1007/BF02174037

Jeddeloh JA, Stokes TL, Richards EJ (1999) Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat Genet 22:94–97. https://doi.org/10.1038/8803

Johannes F, Porcher E, Teixeira FK et al (2009) Assessing the impact of transgenerational epigenetic variation on complex traits. PLoS Genet 5:e1000530. https://doi.org/10.1371/journal.pgen.1000530

Jorgensen R (1993) The germinal inheritance of epigenetic information in plants. Philos Trans R Soc Lond B Biol Sci 339:173–181. https://doi.org/10.1098/rstb.1993.0014

Kakutani T, Jeddeloh JA, Flowers SK et al (1996) Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. Proc Natl Acad Sci 93:12406–12411

King G (1995) Morphological development in Brassica oleracea is modulated by in-vivo treatment with 5-azacytidine. J Hortic Sci 70:333–342

Li Q, Eichten SR, Hermanson PJ et al (2014) Genetic perturbation of the maize methylome. Plant Cell 26:4602–4616. https://doi.org/10.1105/tpc.114.133140

Manning K, Tör M, Poole M et al (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat Genet 38:948–952. https://doi.org/10.1038/ng1841

McClintock B (1958) The suppressor-mutator system of control of gene action in maize. Carnegie Inst Wash Yearb 57:415–429

McClintock B (1965) Components of action of the regulators Spm and Ac. Carnegie Inst Wash Yearb 64:527–536

Miura A, Yonebayashi S, Watanabe K et al (2001) Mobilization of transposons by a mutation abolishing full DNA methylation in Arabidopsis. Nature 411:212–214. https://doi.org/10.1038/35075612

Miura K, Agetsuma M, Kitano H et al (2009) A metastable DWARF1 epigenetic mutant affecting plant stature in rice. Proc Natl Acad Sci 106:11218–11223. https://doi.org/10.1073/pnas.0901942106

Ossowski S, Schneeberger K, Lucas-Lledó JI et al (2010) The rate and molecular spectrum of spontaneous mutations in Arabidopsis thaliana. Science 327:92–94. https://doi.org/10.1126/science.1180677

Patterson GI, Thorpe CJ, Chandler VL (1993) Paramutation, an allelic interaction, is associated with a stable and heritable reduction of transcription of the maize b regulatory gene. Genetics 135:881–894

Patterson GI, Kubo KM, Shroyer T, Chandler VL (1995) Sequences required for paramutation of the maize b gene map to a region containing the promoter and upstream sequences. Genetics 140:1389–1406

Peterson PA (1966) Phase variation of regulatory elements in maize. Genetics 54:249–266

Regulski M, Lu Z, Kendall J et al (2013) The maize methylome influences mRNA splice sites and reveals widespread paramutation-like switches guided by small RNA. Genome Res 23:1651–1662. https://doi.org/10.1101/gr.153510.112

Reinders J, Wulff BBH, Mirouze M et al (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic Arabidopsis epigenomes. Genes Dev 23:939–950. https://doi.org/10.1101/gad.524609

Richards EJ (2006) Inherited epigenetic variation — revisiting soft inheritance. Nat Rev Genet 7:395–401. https://doi.org/10.1038/nrg1834

Roux F, Colomé-Tatché M, Edelist C et al (2011) Genome-wide epigenetic perturbation jump-starts patterns of heritable variation found in nature. Genetics 188:1015–1017. https://doi.org/10.1534/genetics.111.128744

Sano H, Kamada I, Youssefian S et al (1990) A single treatment of rice seedlings with 5-azacytidine induces heritable dwarfism and undermethylation of genomic DNA. Mol Gen Genet MGG 220:441–447. https://doi.org/10.1007/BF00391751

Saze H, Kakutani T (2007) Heritable epigenetic mutation of a transposon-flanked Arabidopsis gene due to lack of the chromatin-remodeling factor DDM1. EMBO J 26:3641–3652. https://doi.org/10.1038/sj.emboj.7601788

Schmitz RJ, He Y, Valdés-López O et al (2013a) Epigenome-wide inheritance of cytosine methylation variants in a recombinant inbred population. Genome Res 23:1663–1674. https://doi.org/10.1101/gr.152538.112

Schmitz RJ, Schultz MD, Urich MA et al (2013b) Patterns of population epigenomic diversity. Nature 495:193–198. https://doi.org/10.1038/nature11968

Silveira AB, Trontin C, Cortijo S et al (2013) Extensive natural epigenetic variation at a de novo originated gene. PLOS Genet 9:e1003437. https://doi.org/10.1371/journal.pgen.1003437

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 15:591–602. https://doi.org/10.1101/gad.193701

Soppe WJJ, Jacobsen SE, Alonso-Blanco C et al (2000) The late flowering phenotype of fwa mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. Mol Cell 6:791–802. https://doi.org/10.1016/S1097-2765(05)00090-0

Springer NM, McGinnis KM (2015) Paramutation in evolution, population genetics and breeding. Semin Cell Dev Biol 44:33–38. https://doi.org/10.1016/j.semcdb.2015.08.010

Stam M, Belele C, Dorweiler JE, Chandler VL (2002) Differential chromatin structure within a tandem array 100 kb upstream of the maize b1 locus is associated with paramutation. Genes Dev 16:1906–1918. https://doi.org/10.1101/gad.1006702

Thompson AJ, Tor M, Barry CS et al (1999) Molecular and genetic characterization of a novel pleiotropic tomato-ripening mutant. Plant Physiol 120:383–390. https://doi.org/10.1104/pp.120.2.383

Vojta A, Dobrinić P, Tadić V et al (2016) Repurposing the CRISPR-Cas9 system for targeted DNA methylation. Nucleic Acids Res 44:5615–5628. https://doi.org/10.1093/nar/gkw159

Vongs A, Kakutani T, Martienssen RA, Richards EJ (1993) Arabidopsis thaliana DNA methylation mutants. Science 260:1926–1928. https://doi.org/10.1126/science.8316832

Waddington CH (1942) The epigenotype. Endeavour 1:18–20

Woo HR, Pontes O, Pikaard CS, Richards EJ (2007) VIM1, a methylcytosine-binding protein required for centromeric heterochromatinization. Genes Dev 21:267–277. https://doi.org/10.1101/gad.1512007

Xu J, Tanino KK, Horner KN, Robinson SJ (2016a) Quantitative trait variation is revealed in a novel hypomethylated population of woodland strawberry (Fragaria vesca). BMC Plant Biol 16:240. https://doi.org/10.1186/s12870-016-0936-8

Xu J, Tanino KK, Robinson SJ (2016b) Stable epigenetic variants selected from an induced hypomethylated Fragaria vesca population. Front Plant Sci 7:1768. https://doi.org/10.3389/fpls.2016.01768