Epigenetic variation and environmental change

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To evaluate if stress-induced epigenetic changes allow plants to adapt to changing environmental conditions, we need to understand target selection and heritability of epigenetic modifications induced by distinct epigenetic pathways.

Abstract

Environmental conditions can change the activity of plant genes via epigenetic effects that alter the competence of genetic information to be expressed. This may provide a powerful strategy for plants to adapt to environmental change. However, as epigenetic changes don't modify DNA sequences and are therefore reversible, only those epi-mutations that are transmitted through the germline can be expected to contribute to a long-term adaptive response. The major challenge for the investigation of epigenetic adaptation theories, is therefore to identify genomic loci that undergo epigenetic changes in response to environmental conditions, which alter their expression in a heritable way and which improve the plant's ability to adapt to the inducing conditions. This article focuses on the role of DNA methylation as a prominent epigenetic mark that controls chromatin conformation, and on its potential in mediating expression changes in response to environmental signals.

Keywords: Epigenetics, DNA methylation, stress response, adaptation

Introduction

Epigenetic mechanisms alter the probability or competence of genetic information to be expressed in a heritable but still reversible way. This is mediated by changes in chromatin structure that alter the accessibility of a genetic region for the transcription machinery, or by changes in turnover rates of selected transcripts. In many, but not all cases, these changes are implemented by small RNAs or longer non coding RNAs that serve as sequence- or locus-specific guides for DNA methylation, chromatin modification or transcript degradation/ amplification mechanisms.

While epigenetic changes can influence mutation and recombination rates, epigenetic target loci do not change their DNA sequence. A local epigenetic modification, as long as it is maintained, therefore alters the conversion of genetic information into a phenotype, while reversal to the original epigenetic state restores the previous *status quo*. This provides plants with an efficient tool to alter gene function in specific cell types, developmental stages or under specific environmental conditions, and to pass on the altered epigenetic state during somatic cell division or even via the germline to subsequent generations. Depending on the epigenetic modification, this can lead to the silencing of a previously active gene or to the activation of a functional but so far silent genetic region. Reversible epigenetic modifications include histone marks, in particular methylation, acetylation or phosphorylation marks at histone tails, and methylation of cytosines. Changes in DNA methylation are the easiest to detect and most precisely positioned indicators and modifiers of epigenetic change, which influence gene expression directly or in combination with histone marks.

DNA methylation pathways in plants

In the model system *Arabidopsis thaliana*, cytosine methylation occurs in three sequence contexts, mediated by DNA methyltransferases that are guided to their targets by methylation patterns, histone marks, small RNAs or non-coding scaffold transcripts. The most prominent methylation marks are found at CG sites, where they are faithfully propagated by maintenance DNA METHYLTRANSFERASE1 (MET1), a plant homolog of the mammalian DNA methyltransferase 1 (Dnmt1), which has a strong affinity for hemi-methylated cytosines. Non-symmetrical cytosine

methylation in a CHH context (H representing C, T or A) is largely controlled by the RNA-directed DNA methylation (RdDM) pathway with 24nt small RNAs (siRNAs) acting as guides for *de novo* DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2). The RdDM pathway predominantly controls repeats in heterochromatic regions and in dispersed transposons, and related sequences in euchromatic regions (Matzke *et al.*, 2009).

Non-coding RNAs and histone marks provide a guiding function for DNA methyltransferases assisting them in identification of their targets. For DRM2-mediated *de novo* methylation this involves two plant-specific RNA polymerases, Pol IV and Pol V, which are only found in plants and which have both evolved from Pol II. Pol IV, which initiates biogenesis of small RNAs, is guided to its target regions by a dual lysine methyl reader protein, DNA-BINDING TRANSCRIPTION FACTOR 1/ SAWADEE HOMEODOMAIN HOMOLOG 1 (DTF1/SHH1), which identifies targets by probing for both unmethylated lysine residues at histone H3 (H3K4) and for methylated H3K9 modifications (Law *et al.*, 2013; Zhang *et al.*, 2013). Pol V, which assists in targeting of the siRNA complex, is guided to its target loci by the DDR chromatin-remodelling complex consisting of DEFECTIVE IN MERISTEM SILENCING 3 (DMS3), DEFECTIVE IN RNA-DIRECTED DNA METHYLATION 1 (DRD1), and RNA-DIRECTED DNA METHYLATION 1 (RDM1) (Zhong *et al.*, 2012) and by two homologues of the histone lysine methyltransferase, *suppressor of variegation 3-9* (SU(VAR)3-9), SUVH2 and SUVH9, with SRA (SET-and RING-ASSOCIATED) domains that bind methylated DNA (Johnson *et al.*, 2014). Pol V assists in the recruitment of DRM2 as part of ARGONAUTE4 (AGO4) effector complexes by producing a non-coding scaffold transcript that base-pairs with siRNAs, which results in specific methylation of the template strand by DRM2 (Zhong *et al.*, 2014) (Figure 1A).

Not all RdDM target loci are controlled by Pol V transcription, as we can distinguish between Pol IV- and Pol V-dependent (type I) loci, and Pol IV-dependent but Pol V-independent (type II) loci. AGO4 co-localises with Pol V in the nucleolar processing centre but not in the nucleoplasm where it associates with Pol II (Gao *et al.*, 2010). Pol II and Pol V therefore have locus-specific AGO4 recruitment functions. Pol II also plays a locus-specific role in siRNA amplification. At intergenic low-copy-number repeat sequences, Pol II produces scaffold transcripts adjacent to silenced loci that

help to recruit Pol V, and Pol II recruits Pol IV to these loci assisting in amplification of siRNA pools (Zheng *et al.*, 2009). The selection of a genomic region as a RdDM target will therefore be influenced by the presence of a pool of homologous siRNAs, by local transcription of scaffold transcripts at or in the vicinity of the locus and by DNA methylation and histone marks at the locus.

A third DNA methyltransferase, CHROMOMETHYLASE3 (CMT3), which is exclusively found in plants, predominantly controls CHG methylation (Jackson *et al.*, 2002) in combination with histone methylation marks (Cao *et al.*, 2003). CMT3 contains a chromodomain that binds methylated H3K9 marks, which are generated by the partially redundant activity of histone methyltransferases SUVH4, SUVH5 AND SUVH6, which contain a methylC binding domain. CHG methylation is therefore maintained by a self-enforcing loop of cytosine and H3K9 methylation enzymes (Johnson *et al.*, 2002). Loss of histone methylation by transcription-associated histone replacement or demethylation (Inagaki *et al.*, 2010) breaks this circle also leading to loss of CHG methylation. At some loci, RdDM pathway functions counter-balance transcription-associated loss of histone methylation and stabilise CMT3-controlled CHG-specific methylation (Enke *et al.*, 2011). Chromomethylases (CMTs) that bind to histone methylation have only been identified in embryophytes (Noy-Malka *et al.*, 2014). Most CMTs analysed so far, including CMT3, preferentially methylate CHG targets. CMT2, however, methylates both CHG and CHH targets (Stroud *et al.*, 2014), acting co-operatively with (Stroud *et al.*, 2014) or independent of the RdDM pathway (Zemach *et al.*, 2013).

The analysis of distinct genomic loci has helped to establish mechanistic models that allocate specific functions to the different DNA methyltransferases. MET1 has mainly been discussed in the context of its maintenance function for CG methylation marks, providing more stable epigenetic patterns than the target loci of the RdDM pathway, which show a higher level of epigenetic variation in *Arabidopsis* accessions (Schmitz *et al.*, 2013). The role of MET1, however, is not strictly limited to maintenance of CG methylation. At least at some target regions, MET1 has been shown to affect non-CG methylation as well, for example as coordinator of methylation of stemloop structures (Gentry and Meyer, 2013) (Figure 1B). An indirect effect on non-CG methylation has been observed at certain loci with CMT2-controlled CHH and CMT3-controlled CHG methylation, which derive from *Gypsy* elements (Figure 1 C and D). These loci lose their H3K9 methylation in a *met1* mutant, which results in a loss of

CHG and CHH methylation marks (Stroud *et al.*, 2013). Loss of MET1 can generate hypomethylated, active epi-alleles, which are stably transmitted to the next generation (Watson *et al.*, 2014).

DNA demethylation pathways in plants

De novo and maintenance methylation in plants is balanced by cytosine demethylation under the control of base excision repair pathways involving the 5-methylcytosine DNA glycosylase REPRESSOR OF SILENCING 1 (ROS1) and its homologs DEMETER (DME), DEMETER-LIKE 2 (DML2), and DML3. After 5mC removal and incision of the DNA backbone, the unmethylated cytosine is restored following 3′ phosphate removal, DNA polymerization and DNA ligation (Penterman *et al.*, 2007). Like DNA methylation, DNA demethylation is linked to histone modification systems, and enzymatic activity and regulation of demethylating complexes is better understood than their target selection criteria. Changes in histone marks are used to recruit demethylation functions or to inhibit *de novo* methylation functions. The histone acetylase Increase DNA Methylation 1 (IDM1), for example, binds to methylated loci with low lysine (H3K4) and arginine (H3R2) methylation levels, and acetylates H3K18 and H3K23 sites to recruit DNA demethylases (Qian *et al.*, 2012). The histone demethylase increase in BONSAI Methylation 1 is recruited to transcribed regions where it demethylates H3K9me2 marks. This removes the binding targets for the chromodomain of CMT3, leading to selective loss of CHG methylation marks that are no longer restored after replication (Inagaki *et al.*, 2010).

In the literature, DNA methylation is often exclusively discussed in the context of gene repression, which does not take into account the complex interaction between the different methylation and demethylation systems. In a *met1* mutant, for example, RdDM functions are activated, while expression of the *ROS1* demethylase is eliminated and *DML2* and *DML3* transcript levels are reduced (Mathieu *et al.*, 2007). Mutation of several RdDM pathway functions also reduces *ROS1* activity (Li *et al.*, 2012), illustrating that the RdDM pathway can also have an activating role via maintaining *ROS1* expression.

Biological effects of DNA methylation

Changes in DNA and histone methylation influence gene expression, in particular transcription (Huettel *et al.*, 2006), splicing (Regulski *et al.*, 2013) and polyadenylation (Tsuchiya and Eulgem, 2013) but they also affect DNA repair (Yao *et al.*), recombination (Mirouze *et al.*, 2012) and meiotic cross-over in euchromatic regions (Melamed-Bessudo and Levy, 2012). The multiple mechanistic effects make it difficult to differentiate between direct changes mediated by DNA methylation and their secondary effects. While the literature is full of reports that correlate DNA methylation and specific phenotypes, there are many fewer reports that demonstrate a direct role of DNA methylation in the transcriptional regulation of one or several distinct target loci, which are responsible for a defined effect or phenotype. Examples of mechanisms and phenotypes under direct control of DNA methylation include parental imprinting (Huh *et al.*, 2008), floral symmetry (Cubas *et al.*, 1999), flowering time (Soppe *et al.*, 2000), pigmentation (Stam *et al.*, 2002), fruit ripening (Manning *et al.*, 2006), sex determination (Martin *et al.*, 2009), and stomatal development (Tricker *et al.*, 2012) (Yamamuro *et al.*, 2014). Seed yield, determined by energy use efficiency, was the first quantitative trait associated with distinct, heritable DNA methylation patterns (Hauben *et al.*, 2009). Flowering time and primary root length are two other complex quantitative traits linked to DNA methylation patterns at differentially methylated regions (DRMs). Methylation patters of some DRMs are heritably altered in epigenetic mutants, which suggest that they are specific targets of an epigenetic system that enhances expression variability. Accordingly, many DRMs display a considerable level of variability in natural *Arabidopsis* populations (Cortijo *et al.*, 2014).

Stress-induced epigenetic changes

While epigenetic *Arabidopsis* mutants have proven useful to test the significance of epigenetic functions in stress responses (Yao *et al.*, 2012) (Popova *et al.*, 2013), we have to be careful when drawing conclusions about a direct role of epigenetic functions especially when using epigenetic mutants that display a range of phenotypes due to secondary effects. Mutation of the *MET1* gene, for example, inhibits expression of DNA demethylases and leads to the establishment of histone H3K9 methylation and RNA-directed methylation marks in new genomic regions (Mathieu *et al.*, 2007). This generates a variety of stochastic epi-mutations and

192 phenotypes, many of which probably don't represent direct MET1 targets but reflect
193 randomly established novel epigenetic marks. Another factor that complicates the
194 comparison of epigenetic mutants and wildtype lines, are background differences in
195 gene expression profiles frequently observed among different plant lines due to
196 epigenetic diversity (Havecker *et al.*, 2012). The use of epigenetic mutants to link
197 phenotypic effects to distinct epigenetic changes, is further complicated by the
198 mutagenic consequence of certain epigenetic alterations, which induce genetic
199 changes that could be mistaken for stable epi-mutations. This is exemplified by the
200 *bal* variant that was isolated from an inbred *ddm1* mutant background and that
201 contains a 55-kb duplication within the *RPP5* (*recognition of Peronospora parasitica*
202 *5*) locus, which includes a cluster of disease *Resistance* (*R*) genes. Duplication is
203 accompanied by hypermutation and up-regulation of *SNC1* (*SUPPRESSOR OF*
204 *NPR1-1*, *CONSTITUTIVE 1*), which co-ordinately activates *RPP5* locus *R* genes and
205 induces a distinct dwarfism and curled-leaf phenotype (Yi and Richards, 2009). It is
206 unclear if these changes represent a random, independent event, or if recombination
207 and mutation rates at the *RPP5* locus are increased by *DDM1* deletion. If
208 hypomethylation induced by mutation of *DDM1* or other methylation functions,
209 stimulates recombination and mutation events at distinct loci, this could lead to
210 genetic changes of identical regions in different DNA methylation mutants that could
211 be mistaken for epi-mutations.
212 To identify direct epigenetic targets for stress effects among a background of epi-
213 alleles and genetic mutations, it will therefore be important to link expression
214 changes at potential epigenetic target loci in epigenetic mutants with corresponding
215 epigenetic changes in response to the stress effect. An example for this strategy is
216 the discovery of epigenetic target loci that are activated in response to bacterial
217 pathogens (Dowen *et al.*, 2012). Indications for a role of DNA methylation in biotic
218 stress responses came from infection studies of methylation mutants *met1-3* and *ddc*
219 (*drm1-2 drm2-2 cmt3-11*), which showed enhanced resistance to pathogenic and
220 avirulent strains of *Pseudomonas syringe*. A screen for differentially methylated
221 regions (DMRs) in wildtype plants, in response to bacterial infection, identified
222 methylation changes at DMRs that correlated with activation of pathogen response
223 genes. While methylation differences were relatively modest due to the high
224 background of unaffected tissue that was not involved in the local response to
225 bacterial infection, they were significant to identify distinct target regions for

pathogen-induced DMRs. These mainly comprised changes in CG and CHH marks in intergenic regions and at 5' and 3' boundaries of protein-coding genes. Infections with virulent and avirulent strains induced similar changes at CG and CHG sites but different changes at CHH sites, which suggest that certain non-symmetrical methylation marks are modified in a stress-specific way. Hypomethylation at non-genic regions correlated with a moderate increase in transcript abundance of proximal genes, while transcript levels were more strongly increased for genes with hypomethylated coding regions. Genes affected by hypomethylation in wildtype after infection, were also misregulated in *met1-3* and *ddc* mutants, which implies that all three methyltransferases were involved in their transcriptional control (Dowen *et al.*, 2012).

Various biotic (Boyko *et al.*, 2007) and abiotic stress conditions (Kovarik *et al.*, 1997) have now been shown to correlate with changes in DNA methylation profiles. We still, however, lack clear evidence for a model case demonstrating that a stress-specific epigenetic modification is transmitted to subsequent generations improving the progeny's capability to cope with the relevant stress (Pecinka and Mittelsten Scheid, 2012). Some reports demonstrate heritable changes in DNA methylation at distinct loci in response to stress but don't show the relevance of these loci to stress tolerance (Kou *et al.*, 2011) (Zheng *et al.*, 2013). Others detect a correlation between stress conditions and overall or tissue-specific methylation changes in putative stress–response genes but don't report on the heritability of these changes (González *et al.*, 2013; Steward *et al.*, 2002). Factors that makes it difficult to assess the relevance of defined epigenetic changes in stress adaptation, are the lack of control over the combined effects of multiple stress conditions a population has been exposed to and the high level of epigenetic variability in populations (Becker *et al.*, 2011; Groszmann *et al.*, 2011; Woo and Richards, 2008).

It is also unclear if epigenetic changes at distinct loci are the direct consequence of changing environmental conditions or if they are the secondary consequences of other stress-induced changes. In this context, it is worth noting that certain environmental stress conditions alter the expression levels of epigenetic regulators. The Geminivirus Rep protein, for example, reduces transcript levels of the *NbMET1* and *NbCMT3* methyltransferase genes in *Nicotiana benthamiana* (Rodríguez-Negrete *et al.*, 2013), and in *Arabidopsis*, *MET1* and *DDM1* transcript levels are down-regulated in response to biotic stress or salicylic acid (Dowen *et al.*, 2012) and

various stress conditions increase transcript levels of histone deacetylases HDA6 (To *et al.*, 2011) and HDA19 (Zhou *et al.*, 2005). At least for certain loci that are sensitive to heritable epigenetic variation in response to environmental conditions, the local concentration of regulatory factors may therefore mediate environmental influences on epigenetic patterns. Environmental effects that alter the concentration of DNA methyltransferases, their interacting histone modifiers or potentially their regulatory siRNA or transcripts (Di Ruscio *et al.*, 2013) (Lakhotia, 2012), may induce epigenetic changes at loci that are sensitive to quantitative changes of key regulators of methylation. Even transient exposure to stress conditions may add to epigenetic diversity if it influences efficiency and fidelity of epigenetic maintenance.

Transposable elements – mediators of epigenetic response

Transposable elements (TEs) and their derivatives, which make up more than half of the DNA in many species, play a prominent role in the epigenetic regulation of adjacent genes, and in the transmission of epigenetic memory effects due to the conversion of epigenetic states in response to environmental change (McClintock, 1984) (Fedoroff, 2012) (Mirouze and Paszkowski, 2011). TEs are controlled by different, frequently interacting epigenetic pathways that determine the stability and fidelity of their transcriptional repression, activation and re-setting (Lippman *et al.*, 2003) (Zemach *et al.*, 2013).

TEs can be activated by stress conditions leading to transient (Tittel-Elmer *et al.*, 2010), cell-specific (Matsunaga *et al.*, 2012) or widespread (Dowen *et al.*, 2012) expression. Activation of TEs can alter expression of adjacent genes and of genes adjacent to new integration sites, into which new TE copies have transposed. Environmental conditions influence the activity of TEs if these contain specific stress-response elements, and they influence the activation of TEs if they change their epigenetic state (Johannes *et al.*, 2009) (McCue *et al.*, 2012). Examples of stress-responsive TEs that insert into genic regions, are *mPing*, a minature inverted-repeat rice TE and the *Arabidopsis ONSEN* retroelement. Amplified copies of *mPing*, which are produced after cold- and salt stress, preferentially insert into 5' regions of genes avoiding potential mutagenic damage via insertion into exons (Naito *et al.*, 2009). *ONSEN* has acquired a heat-responsive element that regulates its activation (Cavrak

292 *et al.*, 2014) and that induces heat-responsiveness in genes adjacent to its new
293 insertion sites (Ito *et al.*, 2011).

294

295 **How useful is an epigenetic stress memory?**

296 The responsiveness of DNA methylation patterns to environmental stress (Finnegan,
297 2002) has been suggested to act as a molecular switch for evolutionary adaptation of
298 plants to environmental change (Kou *et al.*, 2011). In many cases, however, the
299 continuous activity of stress-responsive genes will be undesirable due to secondary
300 effects or the associated energy burden. This may make it advantageous for stress-
301 response pathways with secondary effects to remain active only for the duration of
302 the inducing stress. Under this concept, epigenetic changes should be more useful if
303 they did not cause permanent expression of target genes but if they enabled the
304 gene to respond more quickly and efficiently to frequently re-occurring stress
305 conditions. To detect these kind of epigenetic changes we would face the much
306 harder task of searching for changes in transcriptional competence and/or response
307 time to secondary challenges, not for changes in expression levels.

308 Under continuous stress conditions, it may be advantageous if epigenetic changes
309 lead to continuous activity of stress-response genes that were previously only
310 temporarily active. A potential example where durable changes in environmental
311 conditions could have caused continuous activation of stress-response genes, may
312 be mangrove populations that grow in close vicinity to riverside and salt marsh
313 locations. The two populations differ more significantly in their methylation patterns
314 than in DNA sequence. Plants in the salt marsh population, which display shrub-like
315 phenotypes, have a lower level of methylation diversity than the tree-like plants in the
316 riverside population (Lira-Medeiros *et al.*, 2010). This may reflect a loss of epigenetic
317 flexibility in response to permanent adaptation to salt stress. If this assumption was
318 correct, one would expect to identify active genes in salt marsh populations that are
319 associated with variable methylation patterns in riverside populations, and that are
320 responsible both for improved salt tolerance and changes in plant architecture.

321 While heritable epigenetic changes may be advantageous to adapt to continuous
322 changes in environmental conditions, a transmission of any stress-induced
323 epigenetic state would probably compromise plant growth and development. Plants

have therefore developed several layers of control mechanisms that revert activated epi-alleles to their silent states. Heritability and transmission efficiency of epigenetic patterns are target-specific and dependent on different epigenetic functions. The siRNA pathway plays an important role in restricting retrotransposition triggered by environmental stress. The heat-stress activated *copia*-type *ONSEN* retrotransposon is silenced in in the next generation (Ito *et al.*, 2011) but remains active in plants with compromised siRNA biogenesis. Hypomethylation patterns of RdDM-dependent TEs and their derivatives, are faithfully restored within a few generations (Teixeira *et al.*, 2009) while other hypomethylation patterns are stably retained over at least eight generations (Johannes *et al.*, 2009). DDM1 and Morpheus'Molecule1 (MOM1) have recently been shown to act redundantly to restore silencing of some loci that are activated by heat stress (Iwasaki and Paszkowski, 2014). This does, however, only affect about 10% of all stress-activated genes, which suggests the presence of one or several other resetting mechanisms that prevent trans-generational transmission of epigenetic changes.

Current models and discussions for plants are dominated by the RdDM pathway, and many publications exclusively refer to DNA methylation being established by the guiding function of small RNAs that are generated and transported by RdDM pathway components. While, at least for *Arabidopsis thaliana*, it is certainly correct that DNA methylation of most genomic regions is controlled by the RdDM pathway, we should not ignore the presence of RdDM-independent DNA methylation targets (Gentry and Meyer, 2013; Havecker *et al.*, 2012; Sasaki *et al.*, 2012; Singh *et al.*, 2008; Watson *et al.*, 2014; Zemach *et al.*, 2013). Methylation at some RdDM-independent target loci requires specific epigenetic functions, including HDA6, DDM1 or MET1. These may act as mediators of environmental change if certain stress conditions influence their steady-state levels and if this affects maintenance and stability of their methylation targets.

Outlook

Work on the model system *Arabidopsis thaliana* has helped to define epigenetic pathways, targets and their interactions with various stress conditions. With the rapid completion of genome sequencing projects for various species and the increased

356 resolution of epigenetic maps, we can now investigate species-specific differences in
357 the representation and distribution of epigenetic targets and their control
358 mechanisms. Questions that remain to be answered are: How does a genetic locus
359 become a DNA methylation target and what determines if its DNA methylation
360 pattern is controlled by a RNA-dependent DNA methylation pathway, by a RNA-
361 independent pathway or by a combination of both? Which of these DNA methylation
362 targets produce distinct epi-alleles that are heritable and that contribute to epigenetic
363 diversity? Which of these heritable epigenetic patterns change expression levels and
364 which alter expression competence? Do plant species differ in the composition and
365 representation of target loci for the different DNA methylation pathways, and does
366 this affect their potential to generate epigenetic diversity? How does this influence a
367 plant's potential to cope with stress or to adapt to changing environmental
368 conditions? Considering its relatively low proportion of TEs and TE-derived genes, it
369 is uncertain if *Arabidopsis thaliana* is the best model system to investigate the
370 interplay between epigenetic control of gene activity and a changing environment.
371 We may obtain more relevant examples for epigenetic adaptation from species,
372 which faced gradual changes in their local environment, to which they could respond
373 over several generations, as illustrated by the morphological changes in the
374 mangrove populations mentioned above. Another fascinating example of epigenetic
375 adaptation has been reported for a *Diplacus* species complex in Southern California
376 that changes its flower morphology and colour when adapting to different pollinator
377 populations. Within a geographical transition region containing coastal *Diplacus*
378 *puniceus* plants with red flowers pollinated by hummingbirds and inland *Diplacus*
379 *australis* plants with yellow flowers pollinated by insects, intermediate populations
380 with orange flowers are found. Over a period of 12-15 years, individual plants in this
381 transition zone change in colour and morphology from a yellow, insect-pollinated
382 phenotype to a red bird-pollinated phenotype. The new phenotype is heritable but
383 reverts at a rate of 1-2%, which confirms the epigenetic nature of the morphological
384 change, induced by unknown environmental factors (Hirsch *et al.*, 2012).
385 A search for appropriate epigenetic model systems will help us to assess the
386 significance of epigenetic changes in adaptation to rapidly changing environments,
387 which will ultimately also become highly relevant for the development of novel crops.
388 Considering the historical focus in crop breeding on high yield and uniform
389 development, it is likely that wild plant species have retained a more powerful

epigenetic potential than crop lines – another good reason to rethink the current stringent focus of many research programmes on ‘useful’ species.

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Figure legend:

Figure 1: The role of MET1 in methylation of different target loci in *Arabidopsis thaliana.* Sequence-specific cytosine methylation marks (CG, CNG and CNN) are listed for each DNA methylation function.

MET1 maintains CG methylation marks established by the small RNA pathway (A) but is required for cytosine methylation marks in all sequence contexts in siRNA-independent methylation patterns (B-D). Examples of siRNA-independent methylation are methylation of stem-loop structures that requires coordinated activity of MET1, DRM2 and CMT3, and that depends on the chromatin remodeling protein DRD1 (B), and dense methylation of *Gypsy* elements and their derivatives that requires MET1, CMT2 and CMT3, with (C) or without (D) dependence on the chromatin-remodelling ATPase DDM1.