

## EpiRILs

Catoni, Marco; Cortijo, Sandra

DOI:

[10.1016/bs.abr.2018.08.002](https://doi.org/10.1016/bs.abr.2018.08.002)

License:

None: All rights reserved

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Catoni, M & Cortijo, S 2018, EpiRILs. in *Advances in Botanical Research: Plant Epigenetics Coming of Age for Breeding Applications*. Plant Epigenetics Coming of Age for Breeding Applications, vol. 88, pp. 87-116.  
<https://doi.org/10.1016/bs.abr.2018.08.002>

[Link to publication on Research at Birmingham portal](#)

### **Publisher Rights Statement:**

published in *Advances in Botanical Research*, Vol 88, 2018  
<https://doi.org/10.1016/bs.abr.2018.08.002>

### **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

**Running title: EpiRILs: lessons from Arabidopsis**

## **EpiRILs: lessons from Arabidopsis**

Marco Catoni and Sandra Cortijo

The Sainsbury Laboratory, University of Cambridge, Cambridge, CB2 1LR, UK

Corresponding author: Sandra Cortijo, The Sainsbury Laboratory, 47 Bateman St., Cambridge  
CB2 1LR, UK. Email: [sandra.cortijo@slcu.cam.ac.uk](mailto:sandra.cortijo@slcu.cam.ac.uk)

Keywords: DNA methylation, epiRIL, epialleles, Arabidopsis thaliana, crops, Transposable  
Element

1 **Abstract**

2 In recent times, epigenetic marks have emerged as important players involved in the  
3 regulation of gene expression and transposable element silencing in many organisms. In  
4 plants, many epigenetic changes, mainly at the level of DNA methylation, are  
5 transgenerational stable and contribute to formation of epialleles, affecting developmental  
6 and agronomical traits. In this scenario, it becomes critical to differentiate the genetic from  
7 the epigenetic contribution to plant phenotypes. In Arabidopsis, epigenetic Recombinant  
8 Inbred Lines (epiRILs), obtained by an initial cross of isogenic parents with different DNA  
9 methylation profiles, provide a powerful tool to investigate the role and significance of  
10 epigenetic alteration in identical or almost identical genetic backgrounds. Such populations  
11 have greatly increased our knowledge in mechanisms involved in epialleles formation and  
12 stability, as well as in the consequences of DNA methylation changes in genomic stability,  
13 transposable elements activation and phenotypic traits.

14

## 15 **Introduction**

16

17 While it is known that DNA is the support of heredity, it is more and more recognised that  
18 heritable phenotypic variation can be also caused by epigenetic changes, and not only by  
19 change in the DNA sequence. Methylation of cytosines (DNA methylation) is an epigenetic  
20 mark conserved across many species and plays an important role in regulating gene  
21 expression (X. J. He, Chen, & Zhu, 2011). Widespread perturbation in DNA methylation has  
22 been shown to lead to heritable phenotypic changes in plants (X. J. He et al., 2011; Seymour  
23 & Becker, 2017). Moreover, in plants changes in DNA methylation can be transmitted  
24 through generations because, contrary to what occurs in mammals, there is no clear  
25 evidence of global DNA methylation resetting at each generation (Heard & Martienssen,  
26 2014). DNA methylation in plants occurs at cytosines and can be observed in all three  
27 contexts CG, CHG and CHH. DNA methylation in genes is almost only observed in the CG  
28 context, and seems to be associated with gene activation. On the other hand, dense DNA  
29 methylation at Transposable Elements (TEs) is observed in all three contexts and is  
30 associated with transcriptional repression (Gehring & Henikoff, 2007).

31 To date only a handful of natural epialleles (see definitions) have been described in  
32 eukaryotes (Table 1). These epialleles are characterised by a gain or loss of DNA  
33 methylation, often associated with a change in gene expression, as well as strong  
34 phenotypes. Such changes of DNA methylation at epialleles are usually observed in  
35 repeated sequences or TEs that are either in close proximity or inside genes. While these  
36 epialleles are stable over a generation, some are metastable, which means that some level  
37 of instability as well as gradual reversions have been observed. This is in striking contrast to

38 genetic alleles (see definitions), as changes in the DNA sequence are more stable than  
39 changes in DNA methylation for several orders of magnitude (Becker et al., 2011).  
40 The small number of natural epialleles described so far could be explained by the fact they  
41 were either identified thanks to a strong phenotypes (Bender & Fink, 1995; Cubas, Vincent,  
42 & Coen, 1999; Manning et al., 2006; Martin et al., 2009; Melquist, Luff, & Bender, 1999; K.  
43 Miura et al., 2009; L. Zhang et al., 2012; X. Zhang, Sun, Cao, & Song, 2015), to an allelic  
44 incompatibility between accessions (Agorio et al., 2017; Blevins, Wang, Pflieger, Pontvianne,  
45 & Pikaard, 2017; Durand, Bouche, Perez Strand, Loudet, & Camilleri, 2012) or by chance  
46 (Silveira et al., 2013). We can suppose that many epialleles have not been discovered yet as  
47 they might be associated with mild phenotypes, or phenotypes only visible under certain  
48 circumstances (e.g environmental stress). Moreover, while identifying alleles underlying a  
49 certain phenotype is nowadays straight-forward, identifying DNA methylation changes at an  
50 unknown position associated with a phenotype is still challenging and requires more  
51 sophisticated analysis. In the context of this chapter, we will use a broad definition of  
52 epialleles: any stably transmitted change in methylated profiles, with or without phenotypic  
53 consequences.

54 [Insert Table 1 here]

55 Perturbing DNA methylation, by mutating genes involved in DNA methylation deposition or  
56 maintenance, is a way to increase the chance of detecting epialleles. Hence epialleles have  
57 been detected in *Arabidopsis thaliana* mutants characterised with a global loss of DNA  
58 methylation. Some of these epialleles are characterised by DNA hypomethylation, such as  
59 *fwa*, associated with a late flowering phenotype (Kakutani, 1997; Kakutani, Jeddelloh,  
60 Flowers, Munakata, & Richards, 1996; Lippman et al., 2004; Ronemus, Galbiati, Ticknor,  
61 Chen, & Dellaporta, 1996; Soppe et al., 2000) and *sqn*, associated with an increased

62 expression (Catoni et al., 2017; Habu et al., 2006). Others are characterised by DNA  
63 hypermethylation, such as *sup*, associated with an excess of stamens (Jacobsen &  
64 Meyerowitz, 1997; Jacobsen, Sakai, Finnegan, Cao, & Meyerowitz, 2000), *ag*, associated  
65 with an absence of carpels (Jacobsen et al., 2000) and *bns*, associated with a dwarf  
66 phenotype (Saze & Kakutani, 2007). These epialleles are stably maintained after removal of  
67 the inducible mutation, with a certain degree of metastability, as also observed for natural  
68 epialleles. Except for QQS (Silveira et al., 2013), until now, none of these induced epialleles  
69 have been naturally observed in *Arabidopsis thaliana*.

70 In order to identify alleles with milder or quantitative phenotypes (in contrast to strong  
71 qualitative phenotypes), recombinant inbred lines (RILs, see definitions) are commonly used  
72 (Mackay, 2001). These populations are used to identify loci at which the segregation of  
73 parental alleles are associated with phenotypic changes. Such an approach could also allow  
74 the detection of epialleles associated with mild or quantitative phenotypes. However, as  
75 this will be described in more detail in this chapter, alleles as well as epialleles are  
76 segregating in RIL populations, making it difficult to separate epigenetic from genetic impact  
77 on phenotypes (Johannes, Colot, & Jansen, 2008). In order to specifically identify epialleles  
78 associated with phenotypic changes, epigenetic RIL (epiRILs, see definitions) have been  
79 generated in *Arabidopsis thaliana* (Johannes et al., 2009; Reinders et al., 2009). In short  
80 these populations have been created in order to maximise DNA methylation changes, while  
81 reducing (if not completely removing) DNA sequence differences.

82 In this chapter we will be discussing the many aspects in which epiRIL populations have  
83 been of a great use and how the acquired knowledge could be translated in crops in the  
84 future.

85

86 -----

87 Definitions

88 Allele: Genetic variants of a gene. Different alleles can result in different phenotypic traits.

89 Epiallele: Epigenetic variants of a gene. The genetic sequence of the epialleles is identical,  
90 but the level of DNA methylation, or other epigenetic marks, are different. Epialleles can  
91 result in differences in gene expression, which can potentially lead to differences in  
92 phenotypic traits.

93 RIL (Recombinant Inbred Lines): Set of homozygous lines that incorporate a combination of  
94 genomic regions derived from the cross of two parent lines. Each RIL is developed by self-  
95 pollination and single seed descent propagation of a segregating F2 plant obtained from the  
96 initial cross. Inbreeding continues for at least six/eight generations, determining the fixation  
97 in homozygous form of most of alleles and epialleles. RILs are often used for mapping  
98 QTLs.

99 EpiRIL (Epigenetic Recombinant Inbred Lines): Similarly to RILs, epiRILs are a set of fixed  
100 homozygous lines, descending from a F2 population. However, contrary to RILs, the parents  
101 used to generate epiRIL population have identical (or almost identical) genomic sequence  
102 but different DNA methylation profiles. EpiRILs are thus maximising epialleles segregation,  
103 while reducing (if not removing completely) allelic segregation. EpiRILs can be used for  
104 mapping epiQTLs.

105 QTL (Quantitative Trait Locus): A QTL is a locus of the genome at which genetic variation  
106 correlates with variation of a quantitative trait.

107 EpiQTL (Epigenetic Quantitative Trait Locus): An epiQTL is a locus of the genome at which  
108 variation in DNA methylation correlates with variation of a quantitative trait.

109 Additive alleles: Different alleles of a gene that combine in a way that the phenotype or  
110 expression level of the heterozygous is equal to the sum of each allele.

111 Dominant alleles: The dominant allele dictates the phenotype or expression level of the  
112 heterozygous, when paired with a recessive allele.

113 Transgressive transcripts: In the context of a hybrid, locus expression level that is not  
114 explained neither as additive, nor than as dominant allelic effect.

115 -----

116

### 117 **Definition and description of the epiRIL populations**

118 The study of epiallele stability and phenotypic consequences can be performed by taking  
119 advantage of natural variation in *Arabidopsis thaliana*. DNA methylation at the level of  
120 genes has been shown to be highly polymorphic between *A. thaliana* accessions, making  
121 possible to follow epialleles segregation and their stability in F2 populations (Vaughn et al.,  
122 2007). Natural accessions not only differ in their levels of DNA methylation, but also in their  
123 genetic sequences, and genetic polymorphism can be used to identify the parent of origin  
124 for genomic regions in F1 and F2 populations (Greaves et al., 2012; Shen et al., 2012;  
125 Vaughn et al., 2007; X. Zhang, Shiu, Cal, & Borevitz, 2008).

126 However, the presence of genetic and epigenetic variation across *A. thaliana* natural  
127 accessions often impairs proper quantification of the epigenetic contribution to phenotypic  
128 differences. Indeed, several studies in plants (but also in mammals) reported many  
129 examples of DNA methylation variations associated to either local (*cis*) or distant (*trans*)  
130 changes in DNA sequence (Eichten et al., 2011; Gibbs et al., 2010; Hellman & Chess, 2010; D.  
131 Zhang et al., 2010). On the other hand, mutation rate of methylated cytosines is higher than



132 non methylated cytosines (Xia, Han, & Zhao, 2012), suggesting that DNA methylation and  
133 DNA sequence polymorphisms can be linked and also influence each others.  
134 Therefore, a classification of epialleles has been proposed depending on their link with DNA  
135 sequence polymorphism (Richards, 2006): (i) obligatory epialleles, for which a *cis* or *trans*  
136 genetic polymorphism influences the DNA methylation status; (ii) facilitated epialleles,  
137 which can be linked to or caused by a genetic polymorphism, but that are not fully  
138 dependent on it; and (iii) pure epialleles, that are not affected by any genetic changes.

139

140 Two epiRIL populations have been independently created in *Arabidopsis thaliana* (Figure 1),  
141 to maximise DNA methylation variation and minimise (if not abolishing) DNA sequence  
142 polymorphisms, in order to discriminate epialleles that are not influenced by DNA sequence  
143 polymorphisms (Johannes et al., 2008). These epiRILs have been generated by crossing an  
144 epigenetic mutant, *met1-3* (Reinders et al., 2009) or *ddm1-2* (Johannes et al., 2009), with its  
145 corresponding wild-type (Columbia-0 accession). The two parents thus have the same  
146 genome, except for the mutated gene, but they have very contrasting DNA methylation  
147 profiles. Each epiRIL within the population essentially contains a mosaic epigenome derived  
148 from either wild-type and *ddm1-2* or wild-type and *met1-3*.

149 Although both *met1-3* and *ddm1-2* mutants are hypomethylated, their effects on genome  
150 wide DNA methylation are different, and these differences are conserved in the epigenetic  
151 perturbations segregating in two epiRILs populations. The DNA methyltransferase MET1  
152 maintains CG methylation in *Arabidopsis thaliana* and the *met1-3* null mutant is  
153 characterised by a virtual complete erasure of CG methylation and indirect loss of plant-  
154 specific non-CG methylation (Saze, Mittelsten Scheid, & Paszkowski, 2003). On the other  
155 hand, *DDM1* encodes an ATPase chromatin remodeler primarily involved in allowing DNA

156 methyltransferases to access heterochromatin (Zemach et al., 2013). *ddm1-2* mutation  
157 mainly affects DNA methylation in all cytosine contexts (CG, CHG and CHH) at  
158 heterochromatic TEs (Kakutani, Jeddelloh, & Richards, 1995; Lippman et al., 2004; Vongs,  
159 Kakutani, Martienssen, & Richards, 1993). Consequently, the epialleles generated in *met1-*  
160 derived epiRILs are equally distributed in euchromatic and heterochromatic areas, including  
161 gene bodies that are exclusively CG methylated (Bewick et al., 2016; Catoni et al., 2017),  
162 while epialleles in *ddm1*-derived epiRILs are mostly involving TE loci (Cortijo et al., 2014).  
163 The *met1-3* mutant used to create the *met1*-epiRIL population also shows very severe  
164 phenotypic defects, including reduced fertility (Mathieu, Reinders, Caikovski, Smathajitt, &  
165 Paszkowski, 2007). Hence, a high level of mortality (29%) has also been observed while  
166 propagating 100 individuals of the *met1*-epiRIL population over generations (Reinders et al.,  
167 2009). On the contrary *ddm1*-derived epiRILs have been generated starting from the *ddm1-*  
168 2 mutant, which displays only minor developmental defects. This strategy allowed the  
169 production of a large population of 505 different *ddm1*-derived epiRILs, with no evidence of  
170 selection against deleterious phenotypes (Colome-Tatche et al., 2012).  
171 The crossing scheme of the two populations also differs. In both cases, the mutant (*met1-3*  
172 or *ddm1-2*) has been crossed with a wild-type plant and only F2 plants segregating the wild-  
173 type allele have been used to generate the epiRIL populations. The *met1*-epiRILs originate  
174 directly from the F2 individuals resulting from this cross, while the *ddm1*-epiRILs descend  
175 from a second back cross of the F1 with the wild-type. Thus, DNA methylation changes  
176 segregate with a 1:1 ratio in the *met1*-epiRILs and with a 1:3 (mut/WT) ratio in the *ddm1-*  
177 epiRILs.

178 [Insert Figure 1 here]

179

## 180 DNA methylation transgenerational stability and its phenotypic consequences

181

- 182 • Understanding of the stability of DNA methylation perturbations

183

184 Contrary to mammals, in plants there is no evidence of a consistent global resetting of DNA  
185 methylation during development, making the transmission of epialleles over generations  
186 more probable. Indeed, it has been shown that the loss of DNA methylation induced by the  
187 *ddm1-2* mutation can be stably inherited over many generations once the DDM1 wild-type  
188 allele is re-introduced (Kakutani, Munakata, Richards, & Hirochika, 1999). The analysis of the  
189 transmission of *ddm1-2* and *met1-3* induced hypomethylation at six TEs, after a cross with  
190 wild-type, showed that the hypomethylation is transmitted at some loci and reversed to a  
191 wild-type methylation state at other loci (Lippman et al., 2003). Methylated regions have  
192 been divided into two categories: (i) those that can form two distinct epialleles that are  
193 maintained over generations once in a WT background, and (ii) those that revert to the WT  
194 epigenetic state (remethylatable). EpiRILs are a great tool to study the mechanisms and  
195 consequences of DNA hypomethylation stability or reversion over generations. Indeed, the  
196 analysis of the DNA hypomethylation stability at multiple loci in three *ddm1*-epiRILs and  
197 three *met1*-epiRILs confirmed the presence of stable and remethylatable *ddm1*- and *met1*-  
198 induced hypomethylation (Reinders et al., 2009; Teixeira et al., 2009). Transgressive DNA  
199 methylation patterns have also been observed in these populations. Using bisulfite  
200 sequencing it was shown that remethylation to a level similar to wild-type was observed  
201 occurring at many loci in all cytosine contexts. This remethylation requires sRNA and factors  
202 involved in RNA-directed DNA methylation and is progressive over generations in the *ddm1*-  
203 epiRILs (Teixeira et al., 2009), while remethylation has been observed directly occurring in

204 the F1 in the case of *met1*-induced hypomethylation (Rigal et al., 2016). Further analysis of  
205 *cis* factors influencing remethylation in the *met1*-epiRILs as well as in the F2, containing the  
206 wild-type allele of MET1, originating from a backcross between *met1-3* and wild-type,  
207 showed that remethylation is associated with repetitiveness and relative scarcity of CpGs. In  
208 contrast, stable epialleles are associated with low copy number and high CpG content  
209 (Catoni et al., 2017). The link between these *cis* factors and the level of epigenetic stability  
210 was confirmed in rice (Catoni et al., 2017), and also observed generally associated to the  
211 susceptibility of transgenes to be epigenetically silenced (Sidorenko et al., 2017). This  
212 observation shows how epiRILs in *Arabidopsis thaliana* could be of great help to identify  
213 general rules associated to epiallele stability in different plant species or even for synthetic  
214 or heterologous DNA sequences (like transgenes).

215

- 216 • From epialleles to epigenomic recombination maps

217

218 The identification of epialleles in epiRILs has been used advantageously to identify the  
219 parental origin of genomic regions along the genome, exclusively using DNA methylation  
220 information (Colome-Tatche et al., 2012; Reinders et al., 2009). Parental origin was  
221 identified for three *met1*-epiRILs using whole-genome methylation analysis (Reinders et al.,  
222 2009). Genome-wide DNA methylation data for 123 *ddm1*-epiRILs were also used in order to  
223 construct a recombination map derived from 126 epialleles covering 81.9% of the total  
224 genome (Colome-Tatche et al., 2012). The genetic length of this map is comparable to those  
225 obtained from classical *Arabidopsis* crosses, suggesting that the hypomethylated loci  
226 segregating in the *ddm1*-epiRILs do not affect the global meiotic recombination rates.  
227 However, it has been seen on a local scale that recombination rates are reduced within

228 repeat-rich pericentromeric regions and increased in chromosome arms (Colome-Tatche et  
229 al., 2012). This remodelling of recombination hotspots, without changing the global rate,  
230 was also independently observed using *met1*-epiRILs (Mirouze et al., 2012). A later study  
231 shown that this remodelling of local recombination requires genes involved in the  
232 redistribution of interfering crossovers (Yelina et al., 2015).

233 Interestingly, the creation of epigenomic recombination maps using epialleles has also been  
234 done using mutation accumulation (MA) lines in *Arabidopsis thaliana* (Hofmeister, Lee,  
235 Rohr, Hall, & Schmitz, 2017). MA lines are self-pollinated single-seed descent lines  
236 originating from a single founder, such that the lines are nearly genetically identical. MA  
237 lines display DNA methylation variation, and more than half of the differentially methylated  
238 regions identified in MA lines were stably transmitted in the progeny of a cross between  
239 two of them (Hofmeister et al., 2017). The creation of epigenomic recombination maps  
240 using stable DNA methylation variation is thus not restricted to epiRILs and will be of great  
241 interest to identify epialleles underlying phenotypic variation.

242

- 243 • Epialleles and phenotypic consequences

244

245 Knowing that a proportion of DNA methylation perturbations are transmitted through  
246 generations in *met1* and *ddm1*-epiRILs, one important question is to define if these can have  
247 phenotypic consequences. The two epiRIL populations have been extensively phenotyped  
248 for qualitative as well as quantitative traits such as flowering time, biomass or response to  
249 biotic and abiotic stresses. Two types of phenotypic variation have been observed. A first  
250 type of recessive variation has been observed sporadically occurring in only one epiRIL line  
251 and thus arose specifically during the creation of that line (Figure 2). These specific

252 phenotypic changes are unlikely to be transmitted from the parents used in the creation of  
253 the epiRIL populations, and it was shown that TE mobilisation impairing gene functions were  
254 the cause of such specific phenotypes in the *met1*-epiRIL population (Mirouze et al., 2009).  
255 The second type of phenotypic change is affecting a significant proportion of the epiRIL lines  
256 and is thus potentially inherited from the parents. We will discuss more in detail this second  
257 type of phenotypic change, as they are more likely to be caused by epialleles segregating in  
258 the epiRIL populations.

259 [Insert Figure 2 here]

260 One strong phenotype observed in the two epiRIL populations is delayed flowering time,  
261 which has been shown to be associated with the hypomethylated epiallele at the *FWA* locus  
262 (Johannes et al., 2009; Reinders et al., 2009). However, continuous variation for flowering  
263 time is still observed in the *ddm1*-epiRIL population after removing individuals for which late  
264 flowering is caused by this *fwa* epiallele (Johannes et al., 2009). This suggests that DNA  
265 methylation changes at other loci are also involved in the segregation of this trait in the  
266 *ddm1*-epiRIL population.

267 A large proportion of the *met1*-epiRIL population is also characterised with retarded growth  
268 (85% of *met1*-epiRILs) as well as delayed germination under elevated salinity (60% of *met1*-  
269 epiRILs). Moreover, 34% and 4% of *met1*-epiRILs showed respectively increased resistance  
270 or susceptibility to the biotrophic bacterial pathogen *Pseudomonas syringae* pv. tomato  
271 (Pst) (Reinders et al., 2009).

272 Given the high number of lines in the *ddm1*-epiRIL population (505 lines), many quantitative  
273 traits have been measured in this population and their heritability estimated (i.e. the degree  
274 of variation in the phenotypic trait in the population due to genetic, and here epigenetic,  
275 variation between individuals). A continuous variation and high heritability have been

276 observed for several traits such as flowering time, plant height, primary root length, fruit  
277 number, total biomass and others (Cortijo et al., 2014; Johannes et al., 2009; Roux et al.,  
278 2011). Many traits such as flowering time, plant height, fruit size, dry biomass and rosette  
279 diameter have also been measured in common garden experiments, alongside natural  
280 accessions of *Arabidopsis thaliana* (Roux et al., 2011). It was found that phenotypic  
281 variation in the *ddm1*-epiRIL population displays a level of trait heritability similar to the  
282 natural *Arabidopsis* accessions grown in parallel. Phenotypic plasticity, which is the ability of  
283 one genotype to produce multiple phenotypes in response to the environment, has also  
284 been measured for flowering time, plant height, fruit number, total biomass and root:shoot  
285 ratio in response to drought and nutrient addition (Zhang et al., 2012). A high heritability  
286 was observed for these traits in the absence and presence of environmental perturbations,  
287 but also for their plasticity (Y. Y. Zhang, Fischer, Colot, & Bossdorf, 2013). Theoretical  
288 predictions indicate that these heritability values are consistent with a small number of  
289 parentally derived quantitative trait loci (QTL, see definitions). These results suggest that  
290 phenotypic variability in the *ddm1*-epiRILs can be caused by the segregation of epialleles, or  
291 by DNA sequence polymorphisms caused by mobilisation of transposable elements,  
292 reactivated by DNA hypomethylation.

293 In order to identify the loci underlying heritable phenotypic variability in the *ddm1*-epiRIL  
294 population, and to define their genetic or epigenetic origin, epigenetic quantitative trait loci  
295 (epiQTL, see definitions) have been mapped in *ddm1*-epiRILs for flowering time and primary  
296 root length (Cortijo et al., 2014). This was done taking advantage of a genetic map  
297 generated using differentially methylated regions in 123 *ddm1*-epiRILs, and covering 81.9%  
298 of the total genome (Colome-Tatche et al., 2012). Several epiQTLs were detected on  
299 chromosomes 1, 4 and 5 for flowering time, and on chromosomes 1, 2 and 4 for primary

300 root length (Figure 3). These QTLs could be associated to epigenetic polymorphisms, but  
301 also caused by TE mobilisation. In order to discriminate between these two possibilities,  
302 association between DNA methylation status and primary root length was confirmed for the  
303 markers at the peak of the three epiQTLs in an independent F3 population. Moreover, new  
304 TE mobilisations detected at these epiQTLs in the epiRIL population are not present in this  
305 F3 population. These results strongly suggest that changes in DNA methylation are causing  
306 the epiQTLs detected for primary root length (Cortijo et al., 2014).

307 The next step will be to identify the epialleles underlying these epiQTLs. However, as for  
308 mapping alleles underlying QTLs, this operation is challenging and will require more time  
309 and work. A first step would be to generate a fine mapping population in order to reduce  
310 the size of QTLs and thus the number of potential epialleles (Loudet, Gaudon, Trubuil, &  
311 Daniel-Vedele, 2005). Once potential epialleles will be detected, manipulating their DNA  
312 methylation status will be required in order to confirm the link between DNA methylation  
313 and phenotypic variability at this locus. Targeted DNA methylation is still challenging but  
314 could be now achieved using a deactivated Cas9 fused with a DNA methyltransferase (Vojta  
315 et al., 2016), by VIGS (Bond & Baulcombe, 2015) or by using RNA hairpins to trigger RdDM  
316 (Mette, Aufsatz, van der Winden, Matzke, & Matzke, 2000).

317 However, the complete characterization of epialleles responsible for the identified epiQTL  
318 associated to traits of interest is not necessarily a requirement in order to use this  
319 knowledge to improve plants. Methods such as marker-assisted selection could be used to  
320 introgress the desired trait in the cultivar of interest, taking advantage of markers  
321 associated to the identified epiQTL (Kumar et al., 2017). The DNA methylation status of  
322 these markers, rather than the DNA sequence polymorphisms, would have to be used  
323 during the selection process. Assays based on DNA digestion with enzymes sensitive to DNA



324 methylation, as for example McrBC (Teixeira et al., 2009), associated to qPCR, would provide  
325 a cheap and high throughput approach to perform such selection based on the markers  
326 epigenetic status.

327 [Insert Figure 3 here]

328

### 329 **Using epiRILs to understand TE mobilisation**

330 Transposable elements (TEs) are a heterogeneous group of mobile DNA elements integrated  
331 in the genome of virtually all organisms, with the ability to move from their original position  
332 to a new genomic location. TEs can be classified in two main classes based on their  
333 transposition strategy: (i) Class I TEs (or retrotransposons), which transpose with a copy-  
334 and-paste mechanism through reverse transcription of a RNA intermediate and (ii) Class II  
335 TEs, transposing with a cut-and-paste mechanism mediated by a transposase (Wicker et al.,  
336 2007). Although initially considered as selfish genes and assimilated to “junk DNA” (Doolittle  
337 & Sapienza, 1980), the importance of the contribution of TEs to gene and genome structure  
338 and evolution is currently recognised across the entire tree of life (Hurst & Werren, 2001;  
339 Rebollo, Romanish, & Mager, 2012), including plants (Lisch, 2013). Consequently,  
340 transcriptional silencing of TEs ensures genetic stability, and is controlled in plants by a  
341 network of self-reinforcing epigenetic pathways, marking TEs with repressive marks at the  
342 level of DNA (cytosine methylation) and chromatin (histone repressive marks). Therefore,  
343 epigenetics mutants often show release of TE expression, and have been used to reveal and  
344 study real time TE mobilization (Ito et al., 2011; A. Miura et al., 2001; Tsukahara et al.,  
345 2009). In this context, epiRILs represent a valuable alternative to homozygous *met1*, *ddm1*  
346 and other epigenetic mutants in studying TE mobilization for several reasons. First, epiRILs  
347 are in the wild-type genetic background and are therefore genetically and phenotypically

348 more stable compared to the mutant from which they derived (Reinders et al., 2009).  
349 Moreover, the epiallele segregation and homozygous fixation that occurred through many  
350 inbred generations contributed to “dilute” the epialleles with deleterious effects, reducing  
351 the amount of developmental defects that are normally displayed in the homozygous  
352 mutant. For example, the *Arabidopsis met1-3* mutation is semi-lethal with transgenerational  
353 decrease of fitness, and homozygous mutant plants can be maintained viable for a  
354 maximum of four generations (Mathieu et al., 2007). Although not as severe as for *met1-3*  
355 mutants, *ddm1-1* and *ddm1-2* homozygous mutants accumulate strong phenotypic defects  
356 through generations, (Kakutani et al., 1996). Stochastic bursts of several TEs independently  
357 occur in different *ddm1* inbred lines, and are contributing to at least some of the  
358 developmental phenotypes observed in *ddm1* (A. Miura et al., 2001; Tsukahara et al., 2009).  
359 By contrast, *met1* and *ddm1*-derived epiRILs have been maintained for more than eight  
360 generations without noticing a significant decrease in fertility (Johannes et al., 2009;  
361 Reinders et al., 2009), providing a much more reliable platform to study transposition  
362 events. Indeed, the mobilization of the Class II DNA transposon CACTA1 (Reinders et al.,  
363 2009) and the Class I retrotransposon EVADE (EVD) (Mirouze et al., 2009) were reported in  
364 *met1*-derived epiRILs, while not detected in the *met1-3* mutant. Similarly, many transposons  
365 have been found active in *ddm1*-epiRILs, indicating that *ddm1-2* mutation is necessary to  
366 release TE silencing, and that TEs can remain active after re-introduction of the DDM1 wild-  
367 type allele (Cortijo et al., 2014; Gilly et al., 2014). In *ddm1*-epiRILs the fraction of the  
368 demethylated genome was initially diluted through one *ddm1* backcross of the F1 with the  
369 wild-type, reducing in average to 25% the fraction of hypomethylated genome inherited  
370 from the *ddm1-2* mutant parent, and contributing to stabilize epiRILs phenotypes at late  
371 generations.

372 Therefore, both *met1* and *ddm1* derived epiRIL populations demonstrated a longer  
373 transgenerational viability and stability compared to the mutant parents from which they  
374 are derived. The advantage of this condition is that the plethora of epiallelic effects and  
375 multiple TE activation observed in the homozygous mutants can be isolated in independent  
376 epiRILs, making it possible to study the activation and *de novo* silencing of independent TEs  
377 in real time experiments. For example, the transgenerational dynamic evolution of EVD  
378 mobilization was studied in inbred epiRILs (Mari-Ordonez et al., 2013). The EVD burst and its  
379 *de novo* silencing was reconstructed in a *met1*-epiRIL, observing that efficient silencing is  
380 associated to a change in small RNA composition, and consistently occurs approximately at  
381 the 14th generation after EVD activation, when its copy number in the genome reaches a  
382 threshold of 40 copies (Mari-Ordonez et al., 2013).

383 Although the first events of real time transposition were discovered in maize more than 50  
384 years ago (Mc Clintock, 1950), the impact of TE mobilization on genome stability and the  
385 biology of complex organisms is still poorly investigated, and essentially extrapolated from  
386 comparative genomics and phylogenetic studies. This limitation is the direct consequence of  
387 the rarity of TE mobilization events so far observed in nature, likely due to the epigenetic  
388 silencing normally associated to repeated DNA sequences.

389 The most evident effect of TE mobilization is the recessive mutation of genes with a new TE  
390 insertion occurring in their coding region, in many cases producing a visible phenotype.  
391 Nonetheless, phylogenetic studies produced evidence of several TE-induced non-destructive  
392 effects on gene expression responsible for agricultural important traits in crops (Lisch,  
393 2013). It is however unclear if these non-destructive effects derived from positively selected  
394 exceptional aberrant transposition events or are the result of transposition strategies of  
395 different TE families. In this scenario, epiRILs offer the opportunity to identify and

396 characterize new active TEs, and to study the impact of their real time mobilization across  
397 generations in a limited number of plant lines. Therefore, the study of epiRILs may  
398 contribute to elucidate the role of TE on genetic and biology in higher plants, and more  
399 generally in eukaryotic multicellular organisms.

400

#### 401 **Heterosis**

402 Heterosis, or hybrid vigour, is a phenomenon describing the improved phenotype of a  
403 hybrid offspring compared to the average of both parents, first recorded by Charles Darwin  
404 in 1876 (Darwin, 1876). In agriculture, heterosis has been adopted as a routine strategy for  
405 plant breeding, leading to improved biomass, yield or resistance to biotic and abiotic stimuli  
406 in hybrids (Baranwal, Mikkilineni, Zehr, Tyagi, & Kapoor, 2012). Despite such an extensive  
407 use in agriculture, the underlying mechanisms of heterosis are still poorly understood.  
408 Traditionally, it is generally accepted that heterosis directly correlates with the level of  
409 genetic distance between the two parents (Birchler, Yao, Chudalayandi, Vaiman, & Veitia,  
410 2010). However, more recent experiments performed in *Arabidopsis* have shown that  
411 hybrids generated from accessions with very similar genome can also display a high level of  
412 hybrid vigour (Groszmann, Greaves, Fujimoto, Peacock, & Dennis, 2013; Schneeberger et al.,  
413 2011), suggesting that epigenetic differences could also contribute to heterosis (Figure 4).

414 [Insert Figure 4 here]

415 Indeed, changes in small RNA level and DNA methylation have been associated to hybrid  
416 vigour in both interspecific (i.e. between species) or intraspecific (i.e. between accessions)  
417 hybrids systems studied in *Arabidopsis* (Greaves et al., 2012; Groszmann et al., 2011; Shen  
418 et al., 2012) and other plant species, including rice (Chodavarapu et al., 2012; G. He, He, &  
419 Deng, 2013), maize (Barber et al., 2012; G. He, Chen, et al., 2013), wheat (Kenan-Eichler et

420 al., 2011) and tomato (Shivaprasad, Dunn, Santos, Bassett, & Baulcombe, 2012). However,  
421 the coexistence of genetic and epigenetic differences in hybrids makes it intrinsically  
422 difficult to quantify the epigenetic contribution to heterosis.

423 In contrast, epiRILs are isogenic to wild-type but differ at localized hypomethylated  
424 chromosomal areas. Interestingly, some lines from both *met1*-derived and *ddm1*-derived  
425 epiRIL populations displayed increased biomass or higher resistance to a pathogen if  
426 compared to wild-type Columbia-0 accession (Johannes et al., 2009; Reinders et al., 2009),  
427 similar to what is observed in heterotic hybrids. These results suggest that epigenetic  
428 variation by itself might be involved in the generation of hybrid vigour.

429 In a recent work, heterosis for growth-related traits was investigated in epigenetic hybrids  
430 generated by pollinating *met1*-derived epiRIL plants with pollen from their isogenic wild-  
431 type line (Col-0) (Dapp et al., 2015). In the case of one *met1*-derived epiRIL (epi31), a  
432 consistent and reproducible increase in rosette size was observed in F1 plants compared to  
433 both parental lines. Remarkably, epi31 displayed a clear parent-of-origin effect on hybrid  
434 vigour, as also observed in certain crosses between *Arabidopsis* accessions (Barth, Busimi,  
435 Friedrich Utz, & Melchinger, 2003; Meyer, Torjek, Becher, & Altmann, 2004). Although the  
436 authors could not associate any change in gene expression with the hybrid vigour observed,  
437 several additive, dominant and transgressive (see definitions) transcripts have been identify  
438 in the F1 hybrids (Dapp et al., 2015), supporting the existence of multiple scenarios for DNA  
439 methylation-mediated gene regulation in epi-hybrids.

440 More recently, the contribution of differences in parental methylation to heterosis was  
441 quantified measuring six different traits in a larger panel of over 500 *A. thaliana* epi-hybrids  
442 obtained starting from *ddm1*-derived epiRILs (Lauss et al., 2018). Several positive and  
443 negative heterotic effects were documented, and specific differentially methylated regions

444 in parental genomes were associated with heterotic phenotypes observed in nineteen epi-  
445 hybrids (Lauss et al., 2018).

446 In conclusion, there is growing evidence supporting the epigenetic contribution to heterosis.

447 In this context, epiRILs may be the optimal tool to isolate and characterize epigenetic  
448 determinants of hybrid vigour, for example by mapping epiQTLs associated to different  
449 favourable traits. In addition, altering the epigenetic landscape of parents can potentially  
450 increase the heterotic effect of hybrids, and could be used as a tool to increase plant  
451 productivity.

452

### 453 **Challenges with crops**

454 The investigation of the epigenetic landscape in *Arabidopsis* epiRILs critically contributed to  
455 reveal general plant epigenetic proprieties and mechanisms. Such findings include the  
456 mapping of epiQTLs (Cortijo et al., 2014), the discovery of genetic proprieties that predict  
457 epialleles, common in *Arabidopsis* and rice (Catoni et al., 2017), and a model for origin and  
458 evolutionary consequences of gene body DNA methylation in Angiosperms (Bewick et al.,  
459 2016). However, despite a general conservation of most epigenetic factors and proprieties  
460 across plants, epiRILs are so far only available for *Arabidopsis thaliana*. Creating epiRILs in  
461 crops could improve our understanding of the source of epiallelic creation and also help  
462 detecting epialleles with potential agronomic advantages.

463 The introduction in crops of a level of epigenetic variation similar to that observed in  
464 *Arabidopsis* epiRILs might be of great interest for agriculture. Especially when considering  
465 that crops have larger genomes containing a much higher number of transposons and  
466 repetitive DNA, suggesting an elevated potential for the generation of epialleles.

467 Consistently, rice, maize and tomato mutants in components of epigenetic regulation

468 display strong developmental phenotypes and partial or complete infertility (Gouil &  
469 Baulcombe, 2016; Hu et al., 2014; Li et al., 2014). Remarkably, developmental phenotypes  
470 described in crop epigenetic mutants do not correlate with extensive genome  
471 hypomethylation as observed in *Arabidopsis* (Mathieu et al., 2007), suggesting that in most  
472 plants small perturbations of the methylome have stronger deleterious phenotypic effects  
473 than in *Arabidopsis*.

474 Taking this into account, the generation of crop epiRILs may be impaired by the inability of  
475 producing viable hypomethylated mutants required for the initial cross. However, alternative  
476 strategies should be considered to induce stable epiallele formation without affecting plant  
477 viability (Figure 5).

478 [Insert Figure 5 here]

479 One possibility to reduce genome methylation is the use of hypomethylated partial loss-of-  
480 function epigenetic mutants with mitigated deleterious developmental phenotypes. In  
481 *Arabidopsis*, while the null *met1-3* allele causes complete loss of CpG methylation and is  
482 semi-lethal (Mathieu et al., 2007), the partially functional MET1 protein produced in the  
483 *met1-1* allele can retain CpG methylation in approximately one quarter of the genome,  
484 causing only minor developmental defects and allowing transgenerational conservation of  
485 the *met1-1* mutation in the homozygous form (Kankel et al., 2003). In addition, mobilization  
486 of TEs has also been observed in the *met1-1* mutant background (Griffiths, Catoni, Iwasaki,  
487 & Paszkowski, 2018) as well as the formation of epialleles that are stably maintained for  
488 several generations after transgenic complementation with a wild-type MET1 allele (Catoni  
489 et al., 2017). This suggests that the use of partial loss-of-function mutants might replace null  
490 alleles in epiRIL construction, if a viable knock-out mutant cannot be obtained. However, the  
491 production of partial-loss of function mutants for a chosen gene may be difficult to achieve

492 in plants, and is normally associated to fortuitous screening starting from random  
493 mutagenized populations. Nonetheless, DNA editing strategies, such as CRISPR/ CAS9 (Cong  
494 et al., 2013) and TALEN (Miller et al., 2011) have been successfully extended to plants,  
495 allowing an unprecedented high level of accuracy in targeting chromosomal sequences to  
496 induce mutations (Malzahn, Lowder, & Qi, 2017). Using these approaches, the effect of well  
497 know partial loss-of-function mutations observed in Arabidopsis might be more easily  
498 obtained in the species of interest by targeting a similar mutation in the corresponding  
499 homologous genes.

500 Alternatively, passive DNA hypomethylation has been proposed to occur during  
501 gametogenesis in heterozygous *met1* mutant. The haploid male and female gametophytes  
502 undergo two and three post-meiotic divisions, respectively. Therefore, genomic DNA is  
503 duplicated in gametophytes with the *met1* mutant allele, in absence of the MET1  
504 methylation maintenance system, leading to the passive reduction to 50% and 75% of the  
505 genome methylation respectively in male and female gametes (Saze et al., 2003). This  
506 hypothesis was confirmed by later studies, observing also a genome-wide demethylation  
507 and the formation of stable epialleles in heterozygous inbred *met1* mutant lines, similar to  
508 what was observed in epiRILs (Catoni et al., 2017; Stroud, Greenberg, Feng, Bernatavichute,  
509 & Jacobsen, 2013). Therefore, genome-wide hypomethylation in crop plants may be simply  
510 achieved by inbreeding the usually more fertile heterozygous *met1* mutant, without the  
511 necessity of a viable homozygous mutant allele.

512 One alternative to the generation of epigenetic mutants is the use of drugs interfering with  
513 epigenetic pathways. Inhibitors of DNA methylases, such as 5-Azacytidine and Zebularine,  
514 have been successfully used to induce DNA demethylation in plants (Griffin, Niederhuth, &  
515 Schmitz, 2016; Pecinka & Liu, 2014), including crops (Sano, Kamada, Youssefian, Katsumi, &



516 Wabiko, 1990; Santos et al., 2002; Zhu et al., 2018). Although most of hypomethylation and  
517 transcriptional changes induced by these drugs are only transient (Baubec, Pecinka, Rozhon,  
518 & Mittelsten Scheid, 2009), transgenerational effects have been observed in rice treated  
519 with 5-Azacytidine (Sano et al., 1990). Recently, simultaneous application of Zebularine and  
520 the RNA polymerase II inhibitor  $\alpha$ -amanitin on *Arabidopsis* wild-type seedlings was sufficient  
521 to mobilize the heat-responsive Class I retrotransposon ONSEN, demonstrating that drug  
522 application can efficiently release transposon transcriptional silencing (Thieme et al., 2017).  
523 Finally, another very valuable alternative in order to reduce DNA methylation in plant is the  
524 heterologous expression of enzymes promoting DNA hypomethylation. For example, the  
525 human Ten-eleven translocation (TET) methylcytosine dioxygenases are an enzyme family  
526 catalysing the conversion of 5mC in 5-hydroxymethylcytosine (5hmC), and are involved in  
527 active DNA demethylation in embryonic stem cells (Tahiliani et al., 2009). The transgenic  
528 expression of TET3 catalytic subunit in *Arabidopsis* was enough to decrease DNA  
529 methylation at ribosomal repeats (Hollwey, Watson, & Meyer, 2016). In addition, the  
530 transgenic expression of the same TET3 gene in Tomato induced hypomethylation and  
531 ectopic expression of the CEN1.1 gene in leaves, promoting vegetative growth (Hollwey,  
532 Out, Watson, Heidmann, & Meyer, 2017). In a more recent work, ectopic overexpression of  
533 a different TET gene in *Arabidopsis* induced widespread DNA demethylation and phenotypic  
534 variations, mimicking the effects of *met1* mutation (Ji et al., 2018). In addition, a Cas9-based  
535 targeted demethylation system using the TET1 catalytic subunit was recently generated and  
536 was shown to be able to target demethylation and activate gene expression when directed  
537 to known switchable epialleles in *Arabidopsis* (Gallego-Bartolomé et al., 2018).

538 The combination of these approaches could thus potentially be used in order to promote  
539 global or specific changes in DNA methylation profiles and be the first step to create epiRILs  
540 in crops.

541

## 542 **Conclusion**

543 Arabidopsis epiRIL populations have allowed major advances in understanding the genetic  
544 determinant controlling DNA methylation stability as well as mechanisms involved in the  
545 transgenerational transmission of epigenetic information. Several studies used epiRILs to  
546 highlight the phenotypic consequences of epiallele segregation and the epigenetic  
547 contribution to quantitative traits. While epiRILs have been initially created with the  
548 intention of minimising DNA polymorphisms, the TE reactivation induced by the global loss  
549 of DNA methylation has been used advantageously in order to better understand how TE  
550 mobilisation is controlled, and to study the transgenerational effect of TE activation. EpiRILs  
551 have also helped to better understand the importance of DNA methylation on heterosis,  
552 commonly used in crops to improve yield.

553 The next step to extend the epigenetic potential to improve agricultural traits will be the  
554 creation of epiRILs in crops. This step is challenged by the amount of developmental defects  
555 associated to genome wide hypomethylation observed in epigenetic mutants. Nonetheless,  
556 the better understanding of the epigenetic contribution to phenotypes, and the use of more  
557 sophisticated genome editing strategies might be critical to successfully obtain crop epiRILs  
558 in the near future.

559

## 560 **Acknowledgements**

561 S.C. is supported by the Gatsby foundation, M.C. is supported by European Research Council  
562 (EVOBREED) [322621]. We thank Dr Jayne Griffiths (Sainsbury Laboratory – Cambridge, UK)  
563 for critical reading of the manuscript draft.

564

## 565 **References**

- 566 Agorio, A., Durand, S., Fiume, E., Brousse, C., Gy, I., Simon, M., . . . Bouche, N. (2017). An Arabidopsis  
567 Natural Epiallele Maintained by a Feed-Forward Silencing Loop between Histone and DNA.  
568 *PLoS Genet*, *13*(1), e1006551. doi:10.1371/journal.pgen.1006551
- 569 Baranwal, V. K., Mikkilineni, V., Zehr, U. B., Tyagi, A. K., & Kapoor, S. (2012). Heterosis: emerging  
570 ideas about hybrid vigour. *J Exp Bot*, *63*(18), 6309-6314. doi:10.1093/jxb/ers291
- 571 Barber, W. T., Zhang, W., Win, H., Varala, K. K., Dorweiler, J. E., Hudson, M. E., & Moose, S. P. (2012).  
572 Repeat associated small RNAs vary among parents and following hybridization in maize. *Proc  
573 Natl Acad Sci U S A*, *109*(26), 10444-10449. doi:10.1073/pnas.1202073109
- 574 Barth, S., Busimi, A. K., Friedrich Utz, H., & Melchinger, A. E. (2003). Heterosis for biomass yield and  
575 related traits in five hybrids of Arabidopsis thaliana L. Heynh. *Heredity (Edinb)*, *91*(1), 36-42.  
576 doi:10.1038/sj.hdy.6800276
- 577 Baubec, T., Pecinka, A., Rozhon, W., & Mittelsten Scheid, O. (2009). Effective, homogeneous and  
578 transient interference with cytosine methylation in plant genomic DNA by zebularine. *Plant  
579 J*, *57*(3), 542-554. doi:10.1111/j.1365-313X.2008.03699.x
- 580 Becker, C., Hagmann, J., Muller, J., Koenig, D., Stegle, O., Borgwardt, K., & Weigel, D. (2011).  
581 Spontaneous epigenetic variation in the Arabidopsis thaliana methylome. *Nature*, *480*(7376),  
582 245-249. doi:10.1038/nature10555
- 583 Bender, J., & Fink, G. R. (1995). Epigenetic control of an endogenous gene family is revealed by a  
584 novel blue fluorescent mutant of Arabidopsis. *Cell*, *83*(5), 725-734.
- 585 Bewick, A. J., Ji, L., Niederhuth, C. E., Willing, E. M., Hofmeister, B. T., Shi, X., . . . Schmitz, R. J. (2016).  
586 On the origin and evolutionary consequences of gene body DNA methylation. *Proc Natl Acad  
587 Sci U S A*, *113*(32), 9111-9116. doi:10.1073/pnas.1604666113
- 588 Birchler, J. A., Yao, H., Chudalayandi, S., Vaiman, D., & Veitia, R. A. (2010). Heterosis. *Plant Cell*, *22*(7),  
589 2105-2112. doi:10.1105/tpc.110.076133
- 590 Blevins, T., Wang, J., Pflieger, D., Pontvianne, F., & Pikaard, C. S. (2017). Hybrid incompatibility  
591 caused by an epiallele. *Proc Natl Acad Sci U S A*, *114*(14), 3702-3707.  
592 doi:10.1073/pnas.1700368114
- 593 Bond, D. M., & Baulcombe, D. C. (2015). Epigenetic transitions leading to heritable, RNA-mediated  
594 de novo silencing in Arabidopsis thaliana. *Proc Natl Acad Sci U S A*, *112*(3), 917-922.  
595 doi:10.1073/pnas.1413053112
- 596 Catoni, M., Griffiths, J., Becker, C., Zabet, N. R., Bayon, C., Dapp, M., . . . Paszkowski, J. (2017). DNA  
597 sequence properties that predict susceptibility to epiallelic switching. *Embo j*, *36*(5), 617-  
598 628. doi:10.15252/embj.201695602
- 599 Chodavarapu, R. K., Feng, S., Ding, B., Simon, S. A., Lopez, D., Jia, Y., . . . Pellegrini, M. (2012).  
600 Transcriptome and methylome interactions in rice hybrids. *Proc Natl Acad Sci U S A*, *109*(30),  
601 12040-12045. doi:10.1073/pnas.1209297109
- 602 Colome-Tatche, M., Cortijo, S., Wardenaar, R., Morgado, L., Lahouze, B., Sarazin, A., . . . Johannes, F.  
603 (2012). Features of the Arabidopsis recombination landscape resulting from the combined  
604 loss of sequence variation and DNA methylation. *Proc Natl Acad Sci U S A*, *109*(40), 16240-  
605 16245. doi:10.1073/pnas.1212955109

606 Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., . . . Zhang, F. (2013). Multiplex genome  
607 engineering using CRISPR/Cas systems. *Science*, *339*(6121), 819-823.  
608 doi:10.1126/science.1231143

609 Cortijo, S., Wardenaar, R., Colome-Tatche, M., Gilly, A., Etcheverry, M., Labadie, K., . . . Johannes, F.  
610 (2014). Mapping the epigenetic basis of complex traits. *Science*, *343*(6175), 1145-1148.  
611 doi:10.1126/science.1248127

612 Cubas, P., Vincent, C., & Coen, E. (1999). An epigenetic mutation responsible for natural variation in  
613 floral symmetry. *Nature*, *401*(6749), 157-161. doi:10.1038/43657

614 Dapp, M., Reinders, J., Bediee, A., Balsera, C., Bucher, E., Theiler, G., . . . Paszkowski, J. (2015).  
615 Heterosis and inbreeding depression of epigenetic Arabidopsis hybrids. *Nat Plants*, *1*, 15092.  
616 doi:10.1038/nplants.2015.92

617 Darwin, C. R. (1876). The effects of cross and self fertilisation in the vegetable kingdom. *London:*  
618 *(John Murray)*.

619 Doolittle, W. F., & Sapienza, C. (1980). Selfish genes, the phenotype paradigm and genome  
620 evolution. *Nature*, *284*(5757), 601-603.

621 Durand, S., Bouche, N., Perez Strand, E., Loudet, O., & Camilleri, C. (2012). Rapid establishment of  
622 genetic incompatibility through natural epigenetic variation. *Curr Biol*, *22*(4), 326-331.  
623 doi:10.1016/j.cub.2011.12.054

624 Eichten, S. R., Swanson-Wagner, R. A., Schnable, J. C., Waters, A. J., Hermanson, P. J., Liu, S., . . .  
625 Springer, N. M. (2011). Heritable epigenetic variation among maize inbreds. *PLoS Genet*,  
626 *7*(11), e1002372. doi:10.1371/journal.pgen.1002372

627 Gallego-Bartolomé, J., Gardiner, J., Liu, W., Papikian, A., Ghoshal, B., Kuo, H. Y., . . . Jacobsen, S. E.  
628 (2018). Targeted DNA demethylation of the Arabidopsis genome using the  
629 human TET1 catalytic domain. *Proceedings of the National Academy of Sciences*, *115*(9),  
630 E2125-E2134. doi:10.1073/pnas.1716945115

631 Gehring, M., & Henikoff, S. (2007). DNA methylation dynamics in plant genomes. *Biochim Biophys*  
632 *Acta*, *1769*(5-6), 276-286. doi:10.1016/j.bbaexp.2007.01.009

633 Gibbs, J. R., van der Brug, M. P., Hernandez, D. G., Traynor, B. J., Nalls, M. A., Lai, S. L., . . . Singleton,  
634 A. B. (2010). Abundant quantitative trait loci exist for DNA methylation and gene expression  
635 in human brain. *PLoS Genet*, *6*(5), e1000952. doi:10.1371/journal.pgen.1000952

636 Gilly, A., Etcheverry, M., Madoui, M. A., Guy, J., Quadrana, L., Alberti, A., . . . Aury, J. M. (2014). TE-  
637 Tracker: systematic identification of transposition events through whole-genome  
638 resequencing. *BMC Bioinformatics*, *15*, 377. doi:10.1186/s12859-014-0377-z

639 Gouil, Q., & Baulcombe, D. C. (2016). DNA Methylation Signatures of the Plant  
640 Chromomethyltransferases. *PLoS Genet*, *12*(12), e1006526.  
641 doi:10.1371/journal.pgen.1006526

642 Greaves, I. K., Groszmann, M., Ying, H., Taylor, J. M., Peacock, W. J., & Dennis, E. S. (2012). Trans  
643 chromosomal methylation in Arabidopsis hybrids. *Proc Natl Acad Sci U S A*, *109*(9), 3570-  
644 3575. doi:10.1073/pnas.1201043109

645 Griffin, P. T., Niederhuth, C. E., & Schmitz, R. J. (2016). A Comparative Analysis of 5-Azacytidine- and  
646 Zebularine-Induced DNA Demethylation. *G3 (Bethesda)*, *6*(9), 2773-2780.  
647 doi:10.1534/g3.116.030262

648 Griffiths, J., Catoni, M., Iwasaki, M., & Paszkowski, J. (2018). Sequence-Independent Identification of  
649 Active LTR Retrotransposons in Arabidopsis. *Mol Plant*, *11*(3), 508-511.  
650 doi:10.1016/j.molp.2017.10.012

651 Groszmann, M., Greaves, I. K., Albertyn, Z. I., Scofield, G. N., Peacock, W. J., & Dennis, E. S. (2011).  
652 Changes in 24-nt siRNA levels in Arabidopsis hybrids suggest an epigenetic contribution to  
653 hybrid vigor. *Proc Natl Acad Sci U S A*, *108*(6), 2617-2622. doi:10.1073/pnas.1019217108

654 Groszmann, M., Greaves, I. K., Fujimoto, R., Peacock, W. J., & Dennis, E. S. (2013). The role of  
655 epigenetics in hybrid vigour. *Trends Genet*, *29*(12), 684-690. doi:10.1016/j.tig.2013.07.004

656 Habu, Y., Mathieu, O., Tariq, M., Probst, A. V., Smathajitt, C., Zhu, T., & Paszkowski, J. (2006).  
657 Epigenetic regulation of transcription in intermediate heterochromatin. *EMBO Rep*, 7(12),  
658 1279-1284. doi:10.1038/sj.embor.7400835

659 He, G., Chen, B., Wang, X., Li, X., Li, J., He, H., . . . Deng, X. W. (2013). Conservation and divergence of  
660 transcriptomic and epigenomic variation in maize hybrids. *Genome Biol*, 14(6), R57.  
661 doi:10.1186/gb-2013-14-6-r57

662 He, G., He, H., & Deng, X. W. (2013). Epigenetic variations in plant hybrids and their potential roles in  
663 heterosis. *J Genet Genomics*, 40(5), 205-210. doi:10.1016/j.jgg.2013.03.011

664 He, X. J., Chen, T., & Zhu, J. K. (2011). Regulation and function of DNA methylation in plants and  
665 animals. *Cell Res*, 21(3), 442-465. doi:10.1038/cr.2011.23

666 Heard, E., & Martienssen, R. A. (2014). Transgenerational epigenetic inheritance: myths and  
667 mechanisms. *Cell*, 157(1), 95-109. doi:10.1016/j.cell.2014.02.045

668 Hellman, A., & Chess, A. (2010). Extensive sequence-influenced DNA methylation polymorphism in  
669 the human genome. *Epigenetics Chromatin*, 3(1), 11. doi:10.1186/1756-8935-3-11

670 Hofmeister, B. T., Lee, K., Rohr, N. A., Hall, D. W., & Schmitz, R. J. (2017). Stable inheritance of DNA  
671 methylation allows creation of epigenotype maps and the study of epiallele inheritance  
672 patterns in the absence of genetic variation. *Genome Biol*, 18(1), 155. doi:10.1186/s13059-  
673 017-1288-x

674 Hollwey, E., Out, S., Watson, M. R., Heidmann, I., & Meyer, P. (2017). TET3-mediated demethylation  
675 in tomato activates expression of a CETS gene that stimulates vegetative growth. *Plant*  
676 *Direct*, 1(4), e00022. doi:doi:10.1002/pld3.22

677 Hollwey, E., Watson, M., & Meyer, P. (2016). Expression of the C-Terminal Domain of Mammalian  
678 &TET3 DNA Dioxygenase in <i>Arabidopsis thaliana</i> Induces  
679 Heritable Methylation Changes at <i>rDNA</i> Loci. *Advances in Bioscience and*  
680 *Biotechnology, Vol.07No.05*, 8. doi:10.4236/abb.2016.75023

681 Hu, L., Li, N., Xu, C., Zhong, S., Lin, X., Yang, J., . . . Liu, B. (2014). Mutation of a major CG methylase in  
682 rice causes genome-wide hypomethylation, dysregulated genome expression, and seedling  
683 lethality. *Proc Natl Acad Sci U S A*, 111(29), 10642-10647. doi:10.1073/pnas.1410761111

684 Hurst, G. D., & Werren, J. H. (2001). The role of selfish genetic elements in eukaryotic evolution. *Nat*  
685 *Rev Genet*, 2(8), 597-606. doi:10.1038/35084545

686 Ito, H., Gaubert, H., Bucher, E., Mirouze, M., Vaillant, I., & Paszkowski, J. (2011). An siRNA pathway  
687 prevents transgenerational retrotransposition in plants subjected to stress. *Nature*,  
688 472(7341), 115-119. doi:10.1038/nature09861

689 Jacobsen, S. E., & Meyerowitz, E. M. (1997). Hypermethylated SUPERMAN epigenetic alleles in  
690 arabidopsis. *Science*, 277(5329), 1100-1103.

691 Jacobsen, S. E., Sakai, H., Finnegan, E. J., Cao, X., & Meyerowitz, E. M. (2000). Ectopic  
692 hypermethylation of flower-specific genes in Arabidopsis. *Curr Biol*, 10(4), 179-186.

693 Ji, L., Jordan, W. T., Shi, X., Hu, L., He, C., & Schmitz, R. J. (2018). TET-mediated epimutagenesis of the  
694 Arabidopsis thaliana methylome. *Nat Commun*, 9(1), 895. doi:10.1038/s41467-018-03289-7

695 Johannes, F., Colot, V., & Jansen, R. C. (2008). Epigenome dynamics: a quantitative genetics  
696 perspective. *Nat Rev Genet*, 9(11), 883-890. doi:10.1038/nrg2467

697 Johannes, F., Porcher, E., Teixeira, F. K., Saliba-Colombani, V., Simon, M., Agier, N., . . . Colot, V.  
698 (2009). Assessing the impact of transgenerational epigenetic variation on complex traits.  
699 *PLoS Genet*, 5(6), e1000530. doi:10.1371/journal.pgen.1000530

700 Kakutani, T. (1997). Genetic characterization of late-flowering traits induced by DNA  
701 hypomethylation mutation in Arabidopsis thaliana. *Plant J*, 12(6), 1447-1451.

702 Kakutani, T., Jeddelloh, J. A., Flowers, S. K., Munakata, K., & Richards, E. J. (1996). Developmental  
703 abnormalities and epimutations associated with DNA hypomethylation mutations. *Proc Natl*  
704 *Acad Sci U S A*, 93(22), 12406-12411.

705 Kakutani, T., Jeddelloh, J. A., & Richards, E. J. (1995). Characterization of an Arabidopsis thaliana DNA  
706 hypomethylation mutant. *Nucleic Acids Res*, 23(1), 130-137.

707 Kakutani, T., Munakata, K., Richards, E. J., & Hirochika, H. (1999). Meiotically and mitotically stable  
708 inheritance of DNA hypomethylation induced by ddm1 mutation of *Arabidopsis thaliana*.  
709 *Genetics*, *151*(2), 831-838.

710 Kankel, M. W., Ramsey, D. E., Stokes, T. L., Flowers, S. K., Haag, J. R., Jeddeloh, J. A., . . . Richards, E. J.  
711 (2003). *Arabidopsis* MET1 cytosine methyltransferase mutants. *Genetics*, *163*(3), 1109-1122.

712 Kenan-Eichler, M., Leshkowitz, D., Tal, L., Noor, E., Melamed-Bessudo, C., Feldman, M., & Levy, A. A.  
713 (2011). Wheat hybridization and polyploidization results in deregulation of small RNAs.  
714 *Genetics*, *188*(2), 263-272. doi:10.1534/genetics.111.128348

715 Kumar, J., Gupta, D. S., Gupta, S., Dubey, S., Gupta, P., & Kumar, S. (2017). Quantitative trait loci  
716 from identification to exploitation for crop improvement. *Plant Cell Rep*, *36*(8), 1187-1213.  
717 doi:10.1007/s00299-017-2127-y

718 Lauss, K., Wardenaar, R., Oka, R., van Hulten, M. H. A., Guryev, V., Keurentjes, J. J. B., . . . Johannes,  
719 F. (2018). Parental DNA Methylation States Are Associated with Heterosis in Epigenetic  
720 Hybrids. *Plant Physiol*, *176*(2), 1627-1645. doi:10.1104/pp.17.01054

721 Li, Q., Eichten, S. R., Hermanson, P. J., Zaunbrecher, V. M., Song, J., Wendt, J., . . . Springer, N. M.  
722 (2014). Genetic perturbation of the maize methylome. *Plant Cell*, *26*(12), 4602-4616.  
723 doi:10.1105/tpc.114.133140

724 Lippman, Z., Gendrel, A. V., Black, M., Vaughn, M. W., Dedhia, N., McCombie, W. R., . . . Martienssen,  
725 R. (2004). Role of transposable elements in heterochromatin and epigenetic control. *Nature*,  
726 *430*(6998), 471-476. doi:10.1038/nature02651

727 Lisch, D. (2013). How important are transposons for plant evolution? *Nat Rev Genet*, *14*(1), 49-61.  
728 doi:10.1038/nrg3374

729 Loudet, O., Gaudon, V., Trubuil, A., & Daniel-Vedele, F. (2005). Quantitative trait loci controlling root  
730 growth and architecture in *Arabidopsis thaliana* confirmed by heterogeneous inbred family.  
731 *Theor Appl Genet*, *110*(4), 742-753. doi:10.1007/s00122-004-1900-9

732 Mackay, T. F. (2001). The genetic architecture of quantitative traits. *Annu Rev Genet*, *35*, 303-339.  
733 doi:10.1146/annurev.genet.35.102401.090633

734 Malzahn, A., Lowder, L., & Qi, Y. (2017). Plant genome editing with TALEN and CRISPR. *Cell Biosci*, *7*,  
735 21. doi:10.1186/s13578-017-0148-4

736 Manning, K., Tor, M., Poole, M., Hong, Y., Thompson, A. J., King, G. J., . . . Seymour, G. B. (2006). A  
737 naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor  
738 inhibits tomato fruit ripening. *Nat Genet*, *38*(8), 948-952. doi:10.1038/ng1841

739 Mari-Ordóñez, A., Marchais, A., Etcheverry, M., Martin, A., Colot, V., & Voinnet, O. (2013).  
740 Reconstructing de novo silencing of an active plant retrotransposon. *Nat Genet*, *45*(9), 1029-  
741 1039. doi:10.1038/ng.2703

742 Martin, A., Troadec, C., Boualem, A., Rajab, M., Fernandez, R., Morin, H., . . . Bendahmane, A. (2009).  
743 A transposon-induced epigenetic change leads to sex determination in melon. *Nature*,  
744 *461*(7267), 1135-1138. doi:10.1038/nature08498

745 Mathieu, O., Reinders, J., Caikovski, M., Smathajitt, C., & Paszkowski, J. (2007). Transgenerational  
746 stability of the *Arabidopsis* epigenome is coordinated by CG methylation. *Cell*, *130*(5), 851-  
747 862. doi:10.1016/j.cell.2007.07.007

748 Mc Clintock, B. (1950). The origin and behavior of mutable loci in maize. *Proc Natl Acad Sci U S A*,  
749 *36*(6), 344-355.

750 Melquist, S., Luff, B., & Bender, J. (1999). *Arabidopsis* PAI gene arrangements, cytosine methylation  
751 and expression. *Genetics*, *153*(1), 401-413.

752 Mette, M. F., Aufsatz, W., van der Winden, J., Matzke, M. A., & Matzke, A. J. (2000). Transcriptional  
753 silencing and promoter methylation triggered by double-stranded RNA. *Embo j*, *19*(19),  
754 5194-5201. doi:10.1093/emboj/19.19.5194

755 Meyer, R. C., Torjek, O., Becher, M., & Altmann, T. (2004). Heterosis of biomass production in  
756 *Arabidopsis*. Establishment during early development. *Plant Physiol*, *134*(4), 1813-1823.  
757 doi:10.1104/pp.103.033001

758 Miller, J. C., Tan, S., Qiao, G., Barlow, K. A., Wang, J., Xia, D. F., . . . Rebar, E. J. (2011). A TALE  
759 nuclease architecture for efficient genome editing. *Nat Biotechnol*, *29*(2), 143-148.  
760 doi:10.1038/nbt.1755

761 Mirouze, M., Lieberman-Lazarovich, M., Aversano, R., Bucher, E., Nicolet, J., Reinders, J., &  
762 Paszkowski, J. (2012). Loss of DNA methylation affects the recombination landscape in  
763 Arabidopsis. *Proc Natl Acad Sci U S A*, *109*(15), 5880-5885. doi:10.1073/pnas.1120841109

764 Mirouze, M., Reinders, J., Bucher, E., Nishimura, T., Schneeberger, K., Ossowski, S., . . . Mathieu, O.  
765 (2009). Selective epigenetic control of retrotransposition in Arabidopsis. *Nature*, *461*(7262),  
766 427-430. doi:10.1038/nature08328

767 Miura, A., Yonebayashi, S., Watanabe, K., Toyama, T., Shimada, H., & Kakutani, T. (2001).  
768 Mobilization of transposons by a mutation abolishing full DNA methylation in Arabidopsis.  
769 *Nature*, *411*(6834), 212-214. doi:10.1038/35075612

770 Miura, K., Agetsuma, M., Kitano, H., Yoshimura, A., Matsuoka, M., Jacobsen, S. E., & Ashikari, M.  
771 (2009). A metastable DWARF1 epigenetic mutant affecting plant stature in rice. *Proc Natl*  
772 *Acad Sci U S A*, *106*(27), 11218-11223. doi:10.1073/pnas.0901942106

773 Pecinka, A., & Liu, C. H. (2014). Drugs for plant chromosome and chromatin research. *Cytogenet*  
774 *Genome Res*, *143*(1-3), 51-59. doi:10.1159/000360774

775 Rebollo, R., Romanish, M. T., & Mager, D. L. (2012). Transposable elements: an abundant and natural  
776 source of regulatory sequences for host genes. *Annu Rev Genet*, *46*, 21-42.  
777 doi:10.1146/annurev-genet-110711-155621

778 Reinders, J., Wulff, B. B., Mirouze, M., Mari-Ordonez, A., Dapp, M., Rozhon, W., . . . Paszkowski, J.  
779 (2009). Compromised stability of DNA methylation and transposon immobilization in mosaic  
780 Arabidopsis epigenomes. *Genes Dev*, *23*(8), 939-950. doi:10.1101/gad.524609

781 Richards, E. J. (2006). Inherited epigenetic variation--revisiting soft inheritance. *Nat Rev Genet*, *7*(5),  
782 395-401. doi:10.1038/nrg1834

783 Rigal, M., Becker, C., Pelissier, T., Pogorelnik, R., Devos, J., Ikeda, Y., . . . Mathieu, O. (2016).  
784 Epigenome confrontation triggers immediate reprogramming of DNA methylation and  
785 transposon silencing in Arabidopsis thaliana F1 epihybrids. *Proc Natl Acad Sci U S A*, *113*(14),  
786 E2083-2092. doi:10.1073/pnas.1600672113

787 Ronemus, M. J., Galbiati, M., Ticknor, C., Chen, J., & Dellaporta, S. L. (1996). Demethylation-induced  
788 developmental pleiotropy in Arabidopsis. *Science*, *273*(5275), 654-657.

789 Roux, F., Colome-Tatche, M., Edelist, C., Wardenaar, R., Guerche, P., Hospital, F., . . . Johannes, F.  
790 (2011). Genome-wide epigenetic perturbation jump-starts patterns of heritable variation  
791 found in nature. *Genetics*, *188*(4), 1015-1017. doi:10.1534/genetics.111.128744

792 Sano, H., Kamada, I., Youssefian, S., Katsumi, M., & Wabiko, H. (1990). A single treatment of rice  
793 seedlings with 5-azacytidine induces heritable dwarfism and undermethylation of genomic  
794 DNA. *Molecular and General Genetics MGG*, *220*(3), 441-447. doi:10.1007/bf00391751

795 Santos, A. P., Abranches, R., Stoger, E., Beven, A., Viegas, W., & Shaw, P. J. (2002). The architecture  
796 of interphase chromosomes and gene positioning are altered by changes in DNA methylation  
797 and histone acetylation. *J Cell Sci*, *115*(Pt 23), 4597-4605.

798 Saze, H., & Kakutani, T. (2007). Heritable epigenetic mutation of a transposon-flanked Arabidopsis  
799 gene due to lack of the chromatin-remodeling factor DDM1. *Embo j*, *26*(15), 3641-3652.  
800 doi:10.1038/sj.emboj.7601788

801 Saze, H., Mittelsten Scheid, O., & Paszkowski, J. (2003). Maintenance of CpG methylation is essential  
802 for epigenetic inheritance during plant gametogenesis. *Nat Genet*, *34*(1), 65-69.  
803 doi:10.1038/ng1138

804 Schneeberger, K., Ossowski, S., Ott, F., Klein, J. D., Wang, X., Lanz, C., . . . Weigel, D. (2011).  
805 Reference-guided assembly of four diverse Arabidopsis thaliana genomes. *Proc Natl Acad Sci*  
806 *U S A*, *108*(25), 10249-10254. doi:10.1073/pnas.1107739108

807 Seymour, D. K., & Becker, C. (2017). The causes and consequences of DNA methylome variation in  
808 plants. *Curr Opin Plant Biol*, *36*, 56-63. doi:10.1016/j.pbi.2017.01.005

809 Shen, H., He, H., Li, J., Chen, W., Wang, X., Guo, L., . . . Deng, X. W. (2012). Genome-wide analysis of  
810 DNA methylation and gene expression changes in two Arabidopsis ecotypes and their  
811 reciprocal hybrids. *Plant Cell*, *24*(3), 875-892. doi:10.1105/tpc.111.094870

812 Shivaprasad, P. V., Dunn, R. M., Santos, B. A., Bassett, A., & Baulcombe, D. C. (2012). Extraordinary  
813 transgressive phenotypes of hybrid tomato are influenced by epigenetics and small silencing  
814 RNAs. *Embo j*, *31*(2), 257-266. doi:10.1038/emboj.2011.458

815 Sidorenko, L. V., Lee, T. F., Woosley, A., Moskal, W. A., Bevan, S. A., Merlo, P. A. O., . . . Meyers, B. C.  
816 (2017). GC-rich coding sequences reduce transposon-like, small RNA-mediated transgene  
817 silencing. *Nat Plants*, *3*(11), 875-884. doi:10.1038/s41477-017-0040-6

818 Silveira, A. B., Trontin, C., Cortijo, S., Barau, J., Del Bem, L. E., Loudet, O., . . . Vincentz, M. (2013).  
819 Extensive natural epigenetic variation at a de novo originated gene. *PLoS Genet*, *9*(4),  
820 e1003437. doi:10.1371/journal.pgen.1003437

821 Soppe, W. J., Jacobsen, S. E., Alonso-Blanco, C., Jackson, J. P., Kakutani, T., Koornneef, M., & Peeters,  
822 A. J. (2000). The late flowering phenotype of fwa mutants is caused by gain-of-function  
823 epigenetic alleles of a homeodomain gene. *Mol Cell*, *6*(4), 791-802.

824 Stroud, H., Greenberg, M. V., Feng, S., Bernatavichute, Y. V., & Jacobsen, S. E. (2013). Comprehensive  
825 analysis of silencing mutants reveals complex regulation of the Arabidopsis methylome. *Cell*,  
826 *152*(1-2), 352-364. doi:10.1016/j.cell.2012.10.054

827 Tahiliani, M., Koh, K. P., Shen, Y., Pastor, W. A., Bandukwala, H., Brudno, Y., . . . Rao, A. (2009).  
828 Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL  
829 partner TET1. *Science*, *324*(5929), 930-935. doi:10.1126/science.1170116

830 Teixeira, F. K., Heredia, F., Sarazin, A., Roudier, F., Boccara, M., Ciaudo, C., . . . Colot, V. (2009). A role  
831 for RNAi in the selective correction of DNA methylation defects. *Science*, *323*(5921), 1600-  
832 1604. doi:10.1126/science.1165313

833 Thieme, M., Lanciano, S., Balzergue, S., Daccord, N., Mirouze, M., & Bucher, E. (2017). Inhibition of  
834 RNA polymerase II allows controlled mobilisation of retrotransposons for plant breeding.  
835 *Genome Biol*, *18*(1), 134. doi:10.1186/s13059-017-1265-4

836 Tsukahara, S., Kobayashi, A., Kawabe, A., Mathieu, O., Miura, A., & Kakutani, T. (2009). Bursts of  
837 retrotransposition reproduced in Arabidopsis. *Nature*, *461*(7262), 423-426.  
838 doi:10.1038/nature08351

839 Vaughn, M. W., Tanurdzic, M., Lippman, Z., Jiang, H., Carrasquillo, R., Rabinowicz, P. D., . . .  
840 Martienssen, R. A. (2007). Epigenetic natural variation in Arabidopsis thaliana. *PLoS Biol*,  
841 *5*(7), e174. doi:10.1371/journal.pbio.0050174

842 Vojta, A., Dobrinic, P., Tadic, V., Bockor, L., Korac, P., Julg, B., . . . Zoldos, V. (2016). Repurposing the  
843 CRISPR-Cas9 system for targeted DNA methylation. *Nucleic Acids Res*, *44*(12), 5615-5628.  
844 doi:10.1093/nar/gkw159

845 Vongs, A., Kakutani, T., Martienssen, R. A., & Richards, E. J. (1993). Arabidopsis thaliana DNA  
846 methylation mutants. *Science*, *260*(5116), 1926-1928.

847 Wicker, T., Sabot, F., Hua-Van, A., Bennetzen, J. L., Capy, P., Chalhoub, B., . . . Schulman, A. H. (2007).  
848 A unified classification system for eukaryotic transposable elements. *Nat Rev Genet*, *8*(12),  
849 973-982. doi:10.1038/nrg2165

850 Xia, J., Han, L., & Zhao, Z. (2012). Investigating the relationship of DNA methylation with mutation  
851 rate and allele frequency in the human genome. *BMC Genomics*, *13* Suppl 8, S7.  
852 doi:10.1186/1471-2164-13-s8-s7

853 Yelina, N. E., Lambing, C., Hardcastle, T. J., Zhao, X., Santos, B., & Henderson, I. R. (2015). DNA  
854 methylation epigenetically silences crossover hot spots and controls chromosomal domains  
855 of meiotic recombination in Arabidopsis. *Genes Dev*, *29*(20), 2183-2202.  
856 doi:10.1101/gad.270876.115

857 Zhang, D., Cheng, L., Badner, J. A., Chen, C., Chen, Q., Luo, W., . . . Liu, C. (2010). Genetic control of  
858 individual differences in gene-specific methylation in human brain. *Am J Hum Genet*, *86*(3),  
859 411-419. doi:10.1016/j.ajhg.2010.02.005



860 Zhang, L., Cheng, Z., Qin, R., Qiu, Y., Wang, J. L., Cui, X., . . . Wan, J. (2012). Identification and  
861 characterization of an epi-allele of FIE1 reveals a regulatory linkage between two epigenetic  
862 marks in rice. *Plant Cell*, *24*(11), 4407-4421. doi:10.1105/tpc.112.102269  
863 Zhang, X., Shiu, S. H., Cal, A., & Borevitz, J. O. (2008). Global analysis of genetic, epigenetic and  
864 transcriptional polymorphisms in *Arabidopsis thaliana* using whole genome tiling arrays.  
865 *PLoS Genet*, *4*(3), e1000032. doi:10.1371/journal.pgen.1000032  
866 Zhang, X., Sun, J., Cao, X., & Song, X. (2015). Epigenetic Mutation of RAV6 Affects Leaf Angle and  
867 Seed Size in Rice. *Plant Physiol*, *169*(3), 2118-2128. doi:10.1104/pp.15.00836  
868 Zhang, Y. Y., Fischer, M., Colot, V., & Bossdorf, O. (2013). Epigenetic variation creates potential for  
869 evolution of plant phenotypic plasticity. *New Phytol*, *197*(1), 314-322.  
870 doi:10.1111/nph.12010  
871 Zhu, J., Fang, L., Yu, J., Zhao, Y., Chen, F., & Xia, G. (2018). 5-Azacytidine treatment and TaPBF-D over-  
872 expression increases glutenin accumulation within the wheat grain by hypomethylating the  
873 Glu-1 promoters. *Theor Appl Genet*, *131*(3), 735-746. doi:10.1007/s00122-017-3032-z  
874

875 **Figure legends:**

876 Table 1: Non-exhaustive list of known epialleles in plants.

877

878 Figure 1: Allelic and epiallelic segregation in RIL and epiRIL populations.

879 RIL populations (left) are usually created by crossing two distinct *Arabidopsis* accessions that  
880 are different in their genomes (depicted with different chromosome colours) and  
881 epigenomes (depicted as full or empty dots beside chromosomes). Alleles and epialleles are  
882 thus segregating in F2 population derived by this cross, and fixed in homozygous form by  
883 self-pollination and single seed-descend. By contrast, epiRILs (right) are created by crossing  
884 parents that have identical (or almost identical) genomic sequence but different DNA  
885 methylation profiles. This is obtained in *Arabidopsis* by mutation of *MET1* or *DDM1* genes  
886 (represented by a red horizontal line on chromosome sequence), coding for factors involved  
887 in DNA methylation maintenance. During the generation of epiRILs, only F2 plants with a  
888 *MET1* or *DDM1* wild-type allele are carried out, to avoid new events of genome wide  
889 hypomethylation. EpiRILs are thus maximising epialleles segregation, while reducing (if not  
890 removing completely) allelic segregation.

891

892 Figure 2: Origin of phenotypic changes observed in epiRILs.

893 Phenotypic changes occurring in epiRILs are of two types. The first type (left) is sporadic and  
894 recessive and occurring specifically in one line, probably caused by TE mobilisation or other  
895 genetic mutation. These phenotypes are unlikely to be transmitted from the parents used  
896 in the creation of the epiRIL populations. The second type of phenotypic changes (right)  
897 appears on a significant proportion of epiRIL lines. These traits are potentially inherited from  
898 the parents and likely caused by epialleles segregating in the epiRIL populations.

899

900 Figure 3: Principle of epiQTL mapping in epiRILs for root length, followed by epiallele  
901 identification and validation.

902 In order to identify epiQTLs for a quantitative trait, every line of the population is  
903 phenotyped (top left) and epigenotyped (top right). EpiQTLs are then identified by  
904 measuring the co-segregation of phenotype and epigenotype. Several QTLs were identified  
905 on chromosomes 1, 2 and 4 for root length in the *ddm1*-derived epiRILs (middle). The next  
906 step is to identify epialleles underlying epiQTLs and to validate them by changing their DNA  
907 methylation level (bottom).

908

909 Figure 4: Comparison of epi-hybrid and intraspecies hybrid in *Arabidopsis thaliana*.

910 Examples on enhanced vigour in an epi-hybrid, compared with its two parents, epi31 and  
911 wild-type Col-0 (top), and in an intraspecies hybrid compared to its two parent accessions,  
912 Col-0 and C24 (bottom). In both cases, the epi-hybrid and the intraspecies hybrid are bigger  
913 than their parents, indicating a heterotic effect.

914

915 Figure 5: Different approaches to induce global DNA demethylation in order to create epiRIL  
916 populations.

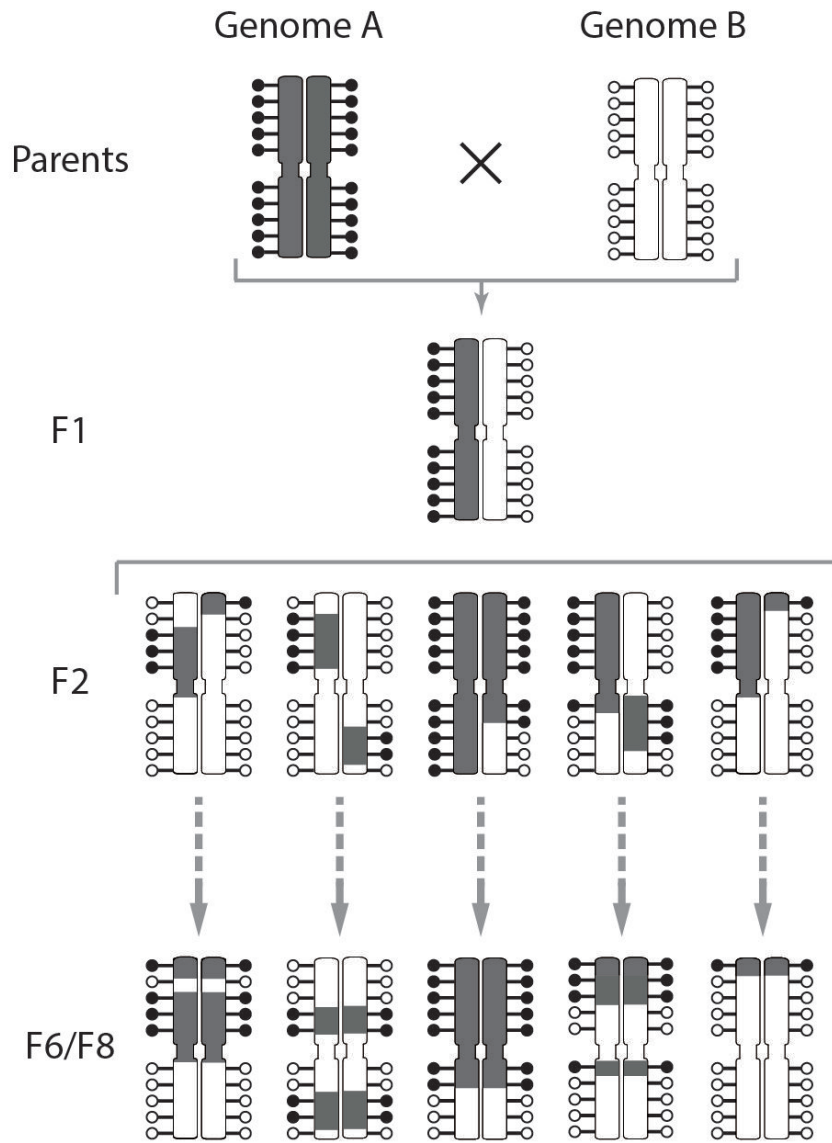
917 In wild-type, DNA maintenance mechanisms ensure conservation of epigenetic marks (i.e.  
918 DNA methylation, represented as black dots). In *met1* or *ddm1* knock-out mutants, DNA  
919 methylation is strongly impaired and normally associated to strong developmental  
920 phenotype. Alternative strategies to reduce DNA methylation limiting the impact on plant  
921 fitness include the use of partial loss-of-function mutations with partial de-methylation; the  
922 self-propagation of heterozygous knock-out mutants, resulting in gametophyte

923 hypomethylation; the application of drugs interfering with methyltransferase activity; and

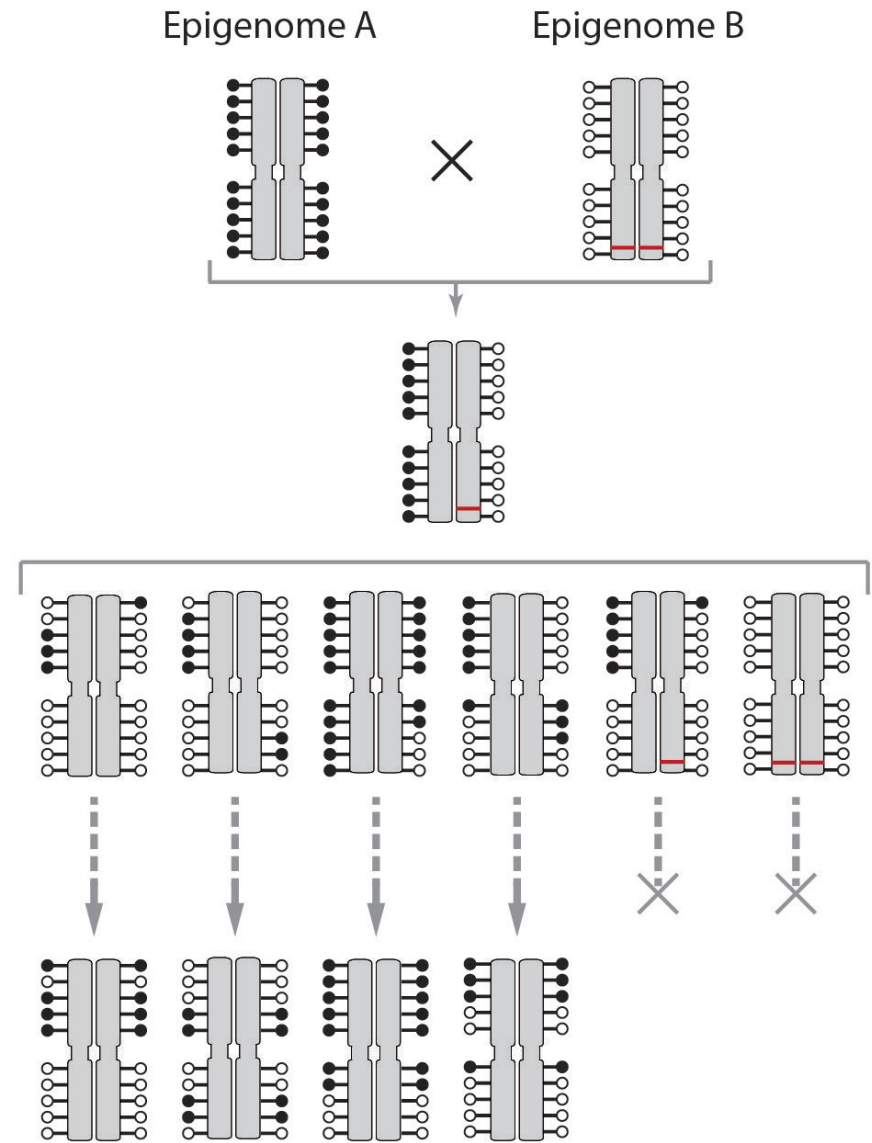
924 the ectopic overexpression of TET methylcytosine dioxygenases.

925

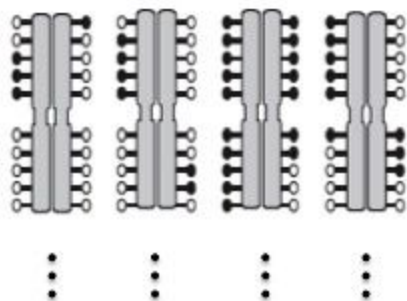
### RIL generation



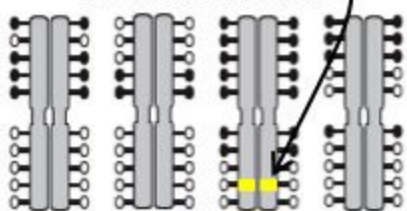
### epiRIL generation



Phenotypic changes  
caused by TE mobilisation

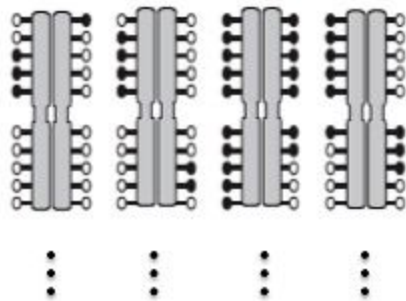


TE mobilization

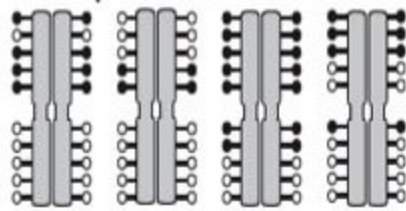


Sporadic phenotype

Phenotypic changes  
caused by epiallele



Epialleles fixation



Segregating phenotype

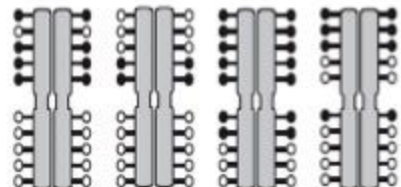
Phenotype

Line 1 Line 2 Line 3 Line 4 ●●●

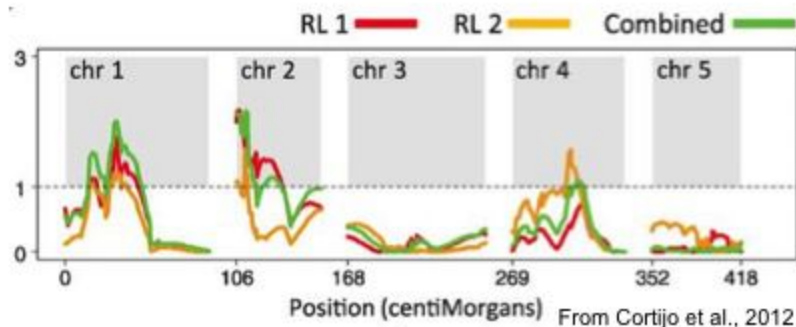


Epigenotype

Line 1 Line 2 Line 3 Line 4 ●●●

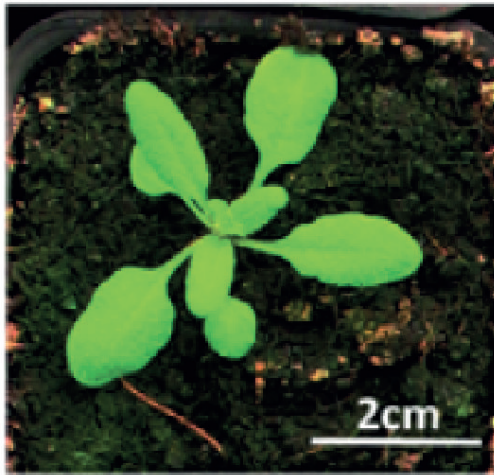


epiQTL mapping



Epiallele identification and validation

WT (Col-0)



epi31 x WT

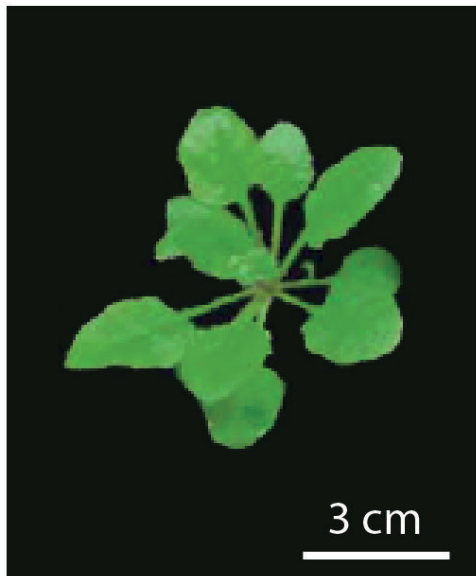


epi31 (Col-0)



From Dapp et al. 2015

Col-0



Col-0 x C24



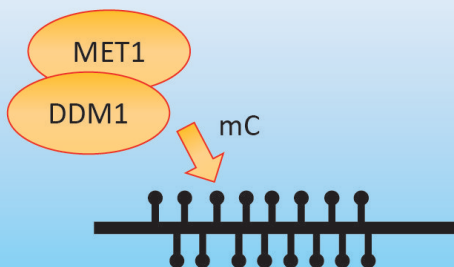
C24



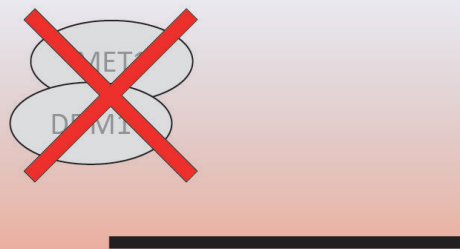
From Chen 2010



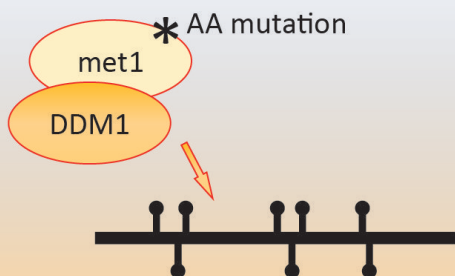
### Wild type



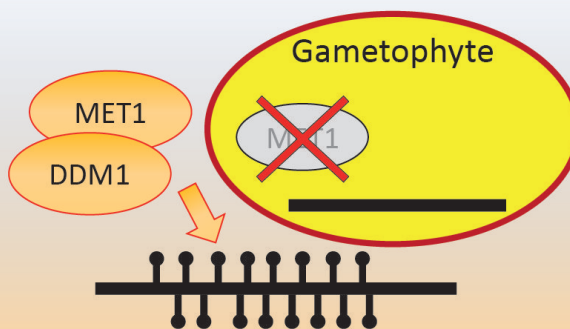
### Knock-out mutants



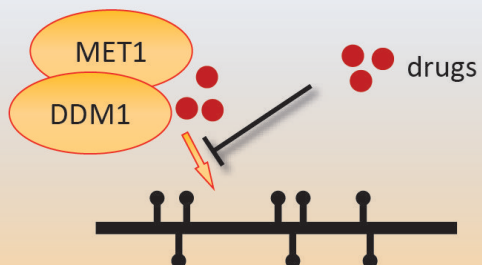
### Partial loss-of-function mutants



### Gametophytic de-methylation



### Methyltransferase inhibitors



### Heterologous enzyme expression

