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Concerning epigenetics and inbreeding

Frank Johannes and Maria Colomé-Tatché

In response to the Review by Charlesworth and Willis (The genetics of inbreeding depression. *Nature Rev. Genet.* **10**, 783–796 (2009))[1], Christian Biémont (Inbreeding effects in the epigenetic era. *Nature Rev. Genet.* **11**, 234 (2010))[2] called for greater attention to be given to the contribution of epigenetic changes to inbreeding depression. To study this phenomenon, the author referred to two recently constructed populations of *Arabidopsis thaliana* epigenetic recombinant inbred lines (epiRILs)[3,4]. The potential of these tools deserves a thorough discussion.

Both epiRIL populations were derived using regular inbreeding techniques (FIG. 1a). The innovative feature is that they originated from two parents with nearly identical genomes but highly divergent epigenomes as a result of a mutation (in one of the parents) in *METHYLTRANSFERASE 1* (*MET1*) or *DECREASE IN DNA METHYLATION 1* (*DDM1*), two genes that are essential for DNA methylation. Plants that are mutant for *met1* or *ddm1* lose over 70% of their DNA methylation, and this loss is in part heritable. The epiRILs segregate both stable and dynamic epigenetic changes[3,4] as well as several nucleotide alterations[3–6] originally induced by the mutations, and can be used for a detailed phenotypic assessment across successive inbreeding generations[7,8].

The *met1*-epiRILs do indeed show severe manifestations of inbreeding depression[3], with about 30% of the lines failing to thrive by generation S7. By contrast, the *ddm1*-epiRILs show greater reproductive fitness (only 0.8% of the lines were lost by S7)[4]. The reason for this discrepancy is unknown, but it should be traceable to essential differences in the type of methylation changes in the parents, the crossing scheme used to derive the epiRILs (backcross versus F_2 base-population), as well as contrasting transposon and genome-wide methylation dynamics during selfing.

Regardless of the underlying causes, the fitness difference between the *met1*-epiRILs and the *ddm1*-epiRILs is not reflected in the inbreeding coefficient[9], which in both cases reaches near unity by generation S7

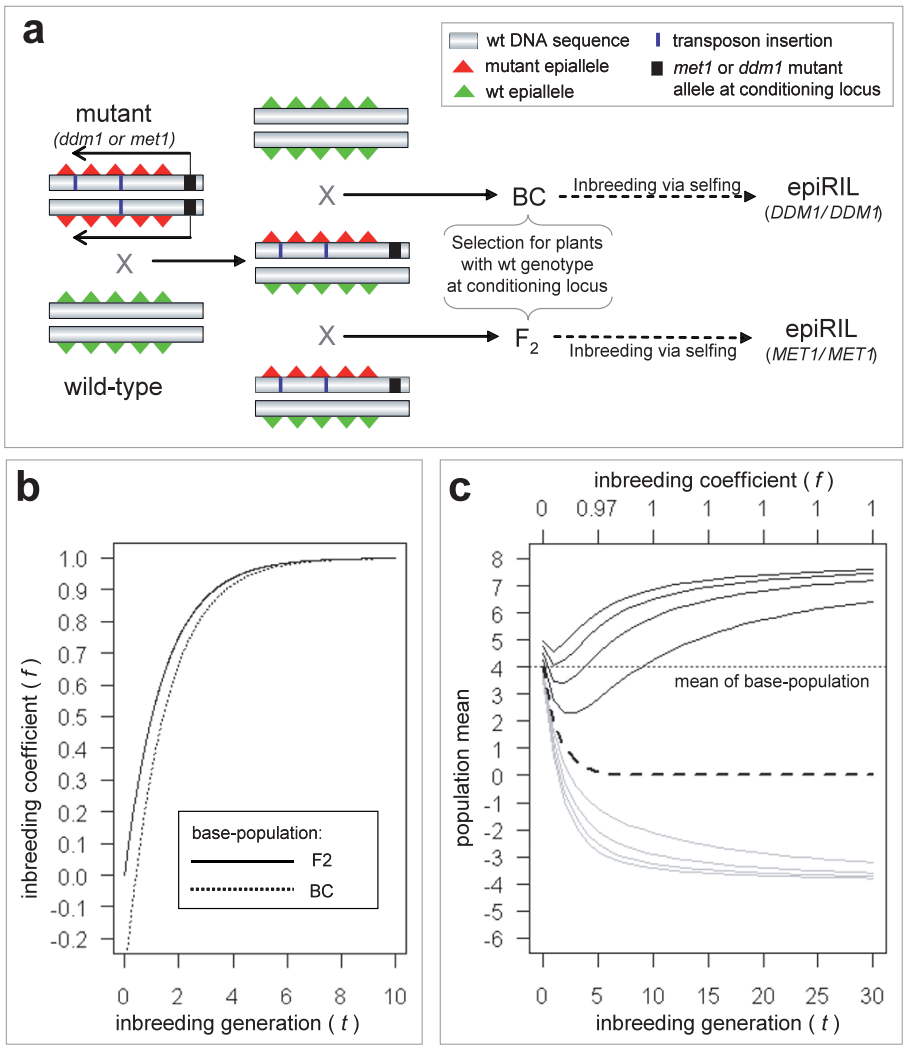

Figure 1 | **EpiRIL construction and inbreeding dynamics. a** | Derivation of the epigenetic recombinant inbred lines (epiRILs) through an initial cross between a wild-type (wt) plant and a *methyltransferase 1* (*met1*) or *decrease in DNA methylation 1* (*ddm1*) mutant plant (wt sequence background). The mutations cause alterations in DNA methylation (indicated by red triangles) as well as remobilization of some transposons (indicated by blue vertical lines). The *ddm1*-epiRLs were derived by selfing individuals from a conditional backcross (BC) (top), and the *met1*-epiRLs from a conditional F_2 (bottom) base-population. **b** | Consider a single locus with wt epiallele *A* and mutant recessive epiallele *a*. Using Wright's method[9] of gametic correlations, it can be shown that for any dynamic changes in epiallele *a*, the inbreeding coefficient increases invariably according to $f_{F_2}(t) = 1 - 2^{-t}$ and $f_{BC}(t) = 1 - 2^{-t}\,4/3$ during selfing for the F_2 and BC cases, respectively. **c** | For comparison, we assume an F_2 base-population only. The plot shows the effect of recurrent inbreeding (*x* axis) on the mean fitness phenotype in the population (*y* axis). In the case of stable alleles with Mendelian inheritance (dashed line), inbreeding occurs according to classical theory. However, when the mutant epiallele *a* progressively deteriorates (for example, as a result of continued loss of methylation (grey lines)), inbreeding depression can occur much more rapidly and depends on the rate of deterioration (reflected in the differences between the grey lines). When the unstable *a* epiallele reverts to the wt state (black lines), inbreeding depression will eventually disappear or even lead to a higher mean fitness phenotype relative to the base-population (dotted line), despite continued inbreeding. The rate of disappearance depends on the rate of reversion to wt (reflected in the differences between the black lines).

(FIG. 1b). This suggests that this classical measure of inbreeding is not adequate for predicting phenotypic depression in these systems. In our opinion, the major challenge in formulating an inbreeding theory for the epiRILs (or similar populations) is to account for dynamic transgenerational changes in DNA methylation in addition to the Mendelian inheritance of parental epialleles[7,10]. Such changes can produce complex phenotypic effects at the population level[7] that simply cannot be accommodated by the classical models reviewed by Charlesworth and Willis.

Consider two extremes of epigenetic dynamics that were previously reported in the epiRILs. The first involves a continued loss of methylation over mutant epialleles to levels outside the parental range[3]. We find that if this process dominates, inbreeding depression advances more rapidly than expected from the inheritance of stable deleterious recessive epialleles (FIG. 1c). By contrast, when causative mutant epialleles progressively revert to wild-type states through RNA-directed remethylation[11,12], inbreeding depression vanishes, despite incremental gains in inbreeding levels (FIG. 1c).

Interestingly, a mechanism akin to this latter process could explain the surge in viability observed by Nebert *et al.*[13] during sibling mating of triple mutant mouse lines, a phenomenon that the authors attributed to epigenetic causes.

The epiRILs inspire a fresh view of quantitative inheritance that combines the transmission of sequence haplotypes according to Mendelian laws with dynamic modifications of chromatin states (epialleles) harboured by these haplotypes[7]. Future modelling efforts should embrace this duality.

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Competing interests statement
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