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The hairy problem of epigenetics in evolution

It has long been suspected that epigenetic mechanisms could contribute to heritable phenotypic variation, and thus to the diversity and evolutionary potential of natural populations (Jablonka & Lamb, 1989; Rapp & Wendel, 2005; Bossdorf *et al.*, 2008). In recognition of the growing evidence bearing on this possibility, the National Evolutionary Synthesis Center (Durham, NC, USA) recently hosted an interdisciplinary gathering of epigeneticists, experimental evolutionary ecologists, behavioural ecologists, theoretical population and quantitative geneticists and philosophers of science to discuss methods available to investigate epigenetic variation and epigenetic inheritance, as well as how to evaluate their importance for phenotypic evolution (Richards *et al.*, 2010a). This vibrant new arena is attracting empirical studies involving natural, nonmodel systems, including those merging robust ecological experimental design with chemical manipulation of genome-wide DNA methylation with 5-azacytidine (Bossdorf *et al.*, 2010) or screening for methylation sensitive amplified fragment length polymorphisms (MS-AFLPs) in response to stress (Verhoeven *et al.*, 2010), different habitats (Herrera & Bazaga, 2010; Paun *et al.*, 2010) and natural levels of herbivory (Herrera & Bazaga, 2011). While these studies have convincingly shown correlations between genome wide changes in methylation with external environment, they ultimately are unsatisfying because surveys such as these do not readily translate into changes in expression of specific genes, particularly those that might be suspected to have ecological relevance. In general, across non-model systems with little or no DNA sequence information, it still is quite challenging to actually demonstrate the specific effects of epigenetic modification on ecologically and evolutionarily relevant phenotypes (Richards *et al.*, 2010b). In this issue of *New Phytologist*, Scoville *et al.* (pp. 251–263) have taken a significant step forward in this direction, identifying a target gene that may be epigenetically modified, adding a remarkable new chapter to the emerging body of work on epigenetic inheritance of trichome density in yellow monkey-flower (*Mimulus guttatus*).


'…*the Scoville* et al. *study may be destined to reside alongside Cubas* et al. *(1999) as seminal in the field.*'

Scoville *et al.* examine the relationship between expression of several candidate genes for trichome development and inheritance of damage-induced trichome production in two parental and four recombinant inbred lines (RILs) of *Mimulus guttatus*. As the authors point out, trichome production is an ideal focal trait for investigating the evolutionary importance of epigenetics because it is a discreet morphological trait that can be induced by environmental stimuli (in some lines), and because the molecular basis has been extensively studied in the model plants *Antirrhinum majus* and *Arabidopsis thaliana*. Consequently, Scoville *et al.* were able to select homologs in *Mimulus* of genes known to be involved in trichome development in snapdragon and arabidopsis. In total, nine candidate trichome homologs were identified in *M. guttatus*, each having sequence similarity to one or more of four *MIXTA*-like transcription factors in *Antirrhinum* or *GL2* in *Arabidopsis*, the latter having all been linked to trichome production.

One of the clear strengths of this study is the ecologically relevant choice of the plant material. The two parental lines have opposite extremes of trichome production that reflects their ecology: the Iron Mountain line (IM767, Fig. 1a) is
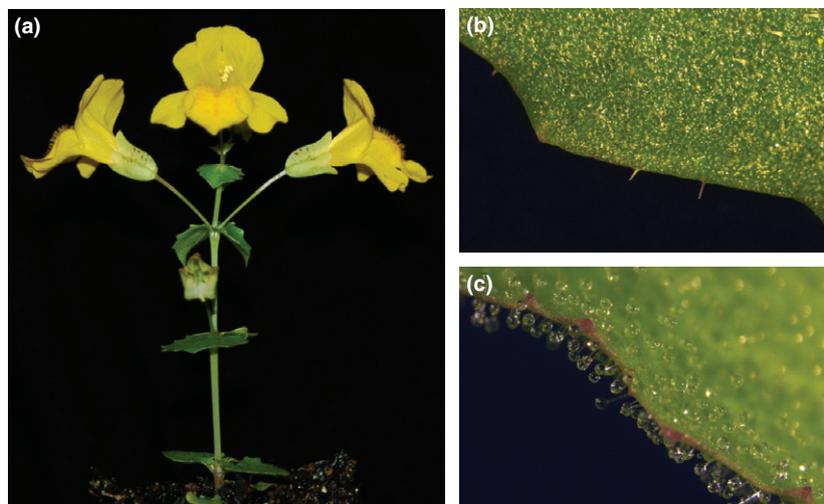
Fig. 1 Variation in trichome density in *Mimulus guttatus*. In the study by Scoville *et al.* (in this issue of *New Phytologist*, pp. 251–263) recombinant inbred lines generated from a cross between *Mimulus guttatus* Iron Mountain 767 (IM767; a,b) and *M. guttatus* Point Reyes (PR; c) were used to demonstrate that expression of a MYB *MIXTA*-like transcription factor is correlated with epigenetic inheritance of trichome density. *Mimulus guttatus* IM767 plants develop leaves with few, generally nonglandular trichomes (b). *Mimulus guttatus* PR plants develop leaves with many, glandular, trichomes (c). Images courtesy of Lena Hileman.

from a population in the Cascade Mountains that does not experience much herbivory and typically produces few trichomes (Fig. 1b), while the coastal Point Reyes (PR, Fig. 1c) line is from a population that experiences high levels of herbivory and has high trichome density. While trichome density appears to be constitutive in the case of these parental lines, the RILs chosen for study have intermediate levels of initially *inducible* levels of trichome production. In a previous study (and again in the current study), several of these lines showed increased trichome density in offspring of artificially damaged parents, as well as genetic variation in the inheritance of parental damage (Holeski, 2007).

The study of Scoville *et al.* is remarkable because it links an ecologically sensitive phenotype to an epigenetically conditioned and inherited cause, and because epigenetic inheritance of increased trichome density is linked to a particular *MYB* transcription factor. The only other clear example of such an ecologically important, epigenetically inherited phenotype that is related to a specific gene is the celebrated study by Cubas *et al.* (1999), who showed that the naturally occurring change from normal bilateral to radial symmetry in the perianth of flowers of *Linaria vulgaris* was associated with methylation and silencing of the single gene *Lcyc*. This example has stood as the poster child of the potential importance of epigenetic changes, but even though this discovery traces to the dawn of the genomics era, additional examples have remained elusive. There are few examples of the phenotypic effects of epigenetic alterations of one or a few genes, especially involving changes that are stably inherited for generations. Because of this linkage between ecological relevance, epigenetic stability and inheritance, and genetic basis, the Scoville *et al.* study may be

destined to reside alongside Cubas *et al.* (1999) as seminal in the field.

The implications of the study of Scoville *et al.* are profound, introducing as it does this additional layer of ecologically relevant epigenetic complexity into considerations of the evolutionary process. Notably, there is variation in the penetrance of epigenetic inheritance among lines, suggesting that the trait of epigenetic inheritance is itself variable and hence potentially visible to selection. In addition, the authors show that there are interactions between line effects and epigenetic effects. While the study offers a fascinating advance in our understanding of the potential ecological and evolutionary significance of epigenetic regulation, it represents just an initial first step, one that raises more questions than provides answers. One can envision numerous follow-up experiments, using a variety of approaches and perspectives. For example, conducting *in situ* hybridization studies with the *MgMYBML8* that putatively is involved in the epigenetically-mediated increase in trichome density would reveal the specific location and duration of gene expression during leaf and trichome development, and how that varies among the different lines. Cubas *et al.* (1999) used this approach in *Linaria* to demonstrate early expression of *Lcyc* in the dorsal region of floral meristems in wild type, but no expression in the peloric mutant.

Besides exploring the specific functions of *MgMYBML8*, the relevance of this gene was inferred using a translational, candidate gene approach instead of an 'uninformed', more global screen of gene expression. Accordingly, further insight into the epigenetic contribution to inheritance of trichome production is likely to derive from more global gene sampling approaches (e.g. RNAseq). The extraordinary power of next-

generation sequencing can be harnessed to study differential expression of *all* genes during trichome development, using tissues of various ages, lines, and growing conditions. This approach may be especially informative when combined with other advanced molecular technologies for detecting epigenetic change, such as chromatin immunoprecipitation against various epigenetic marks, genome-scale sequencing of methylated DNA, and genomic tiling arrays (Park, 2009; Harris *et al.*, 2010). These approaches have the potential to reveal in exquisite detail the network of expression partners involved in the epigenetically mediated expression cascade.

In addition to exploring genome wide involvement in trichome response, a great deal remains to be learned about the scope and duration of the epigenetic response and the noted variability in genetic differences among lines in epigenetic potential. As the authors suggest, it would be informative to conduct multi-generational investigations of the epigenetic response to assess the trans-generational persistence of the phenomenon beyond the two generations presented in the current study. Given the reported variation that exists among lines and populations in epigenetic potential, this kind of information on 'persistence' would inform our understanding of both underlying mechanism and evolutionary significance, particularly inasmuch as the epigenetic capability may have genetically variable underpinnings and may be visible to natural selection operating at the level of resistance to herbivory. Logical extensions include questions about the basis of the genetic difference among lines, whether the phenomenon applies to other populations of *Mimulus guttatus*, other species of *Mimulus*, and other angiosperms in which constitutive expression of trichomes has been implicated as an important component of the defense arsenal.

Among the many advantages of the *Mimulus* system is that a draft genome sequence has been assembled, potentially allowing for an understanding of the control of *MgMYBML8*, and whether it is regulated in *trans*, *cis* or some combination of the two. Forward genetic approaches might be particularly revealing in this regard, where, for example the recombinant inbred lines used could be extensively mapped and compared to the parental lines to generate deeper insight into the expression quantitative trait loci (eQTLs) that underlie *MgMYBML8* levels. These are exciting prospects, which when applied to the extraordinary ecological and morphological diversity within natural monkeyflower populations, hold great promise for a true integration across scales of biological organization and new insight into the ecological genetics of adaptation.

At this time in the science of epigenetics, the contribution of heritable epigenetic effects to adaptation in most organisms is still highly speculative. The study by Scoville *et al.* provides one of the first glimpses into how epigenetic variation can affect an ecologically important trait. We look forward to future studies that further explore the relationships between genetic, epigenetic and phenotypic variation.

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