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Morphological Evolution: By Any Means Necessary?

Recent debate has focused on the role of *cis*-regulatory mutations in the evolution of genes controlling morphology. Identification of the molecular basis of naturally occurring variation in leaf hair (trichome) density in *Arabidopsis*, combined with earlier work in the same system, sheds light on this debate.

John C. Larkin

> "For myself, I … compare evolutionary processes to that most pragmatic of engineers, a teenager with a junk car (thus we may expect to find in the molecular record both chewing gum and baling wire)."
>
> — Elizabeth C. Raff, in [1].

One unexpected discovery following from the reunion of developmental biology with both classical genetics and evolutionary biology was the realization that the function of transcription factors controlling key steps in development is often conserved over long evolutionary timescales; indeed, this was a key insight involved in creating the field of evolutionary developmental biology (evo–devo). For example, the mouse homolog of the *Drosophila eyeless* gene, a master switch gene in eye development, can direct the formation of eyes when expressed in *Drosophila* [2]. At the same time, it became apparent that *cis*-regulatory regions, the DNA sequences adjacent to coding regions that contain binding sites for regulatory transcription factors and that govern tissue-specific expression of key regulators, were often both complex and modular. These observations led to a model stemming from the evo–devo community whereby evolution of morphological traits was proposed to proceed preferentially via changes in *cis*-acting regulatory sequences [3]. This model of evolution contrasted sharply with the more prevailing view that evolution occurs primarily by gene duplication followed by functional divergence [4], with no special role presumed for *cis*-elements relative to changes in the encoded amino acid sequences. Several lines of reasoning were proposed justifying a privileged role for *cis*-elements. These include the observation that proteins controlling development often participate independently in several distinct developmental processes, a property termed pleiotropy, and thus mutations that would be favorable in the context of one process might likely be selected against due to disruption of another process. It was also proposed that the modular nature of *cis*-regulatory sequences controlling expression of developmental regulators would allow functional divergence without gene duplication. This view of molecular evolution has led to a lively debate in the literature, and the model has been attacked [5] and defended [6,7] both on the basis of specific examples and on theoretical grounds. At this point, new examples of mutations affecting developmental processes are welcome, especially if they are initially identified without bias toward any particular evolutionary model.

One such example is provided in a report by Hilscher *et al.* [8] in this issue of *Current Biology*. The authors identify a mutation responsible for naturally occurring variation in the density of shoot epidermal hairs (trichomes) on leaves of the plant *Arabidopsis thaliana*. *Arabidopsis* trichomes have been shown to protect the plants against insect herbivores [9], though they may play a role in protecting the plants from abiotic stress as well. Trichome development is controlled by a transcription factor complex consisting of the MYB protein GL1, the basic helix-loop-helix protein GL3, and the WD40-repeat protein TTG [10]. In addition, several variant MYB proteins incapable of activating transcription compete with GL1 for binding to GL3, thus inhibiting trichome development. These inhibitory MYBs play a role in controlling the spacing of trichomes on leaves. The ETC2 protein is one such inhibitory MYB [11]. Hilscher *et al.* [8] show that the variation in trichome density between the Can-0 (Figure 1A) and Gr-1 (Figure 1B) *Arabidopsis* lines is due to an amino acid change in the protein ETC2. Thus, the low trichome density line Gr-1 actually has higher ETC2 function. This pair of allelic variants maps to the same locus as a quantitative trait for trichome density identified in a different pair of *Arabidopsis* strains, Col and Ler, more than a dozen years ago [12]. The present work now shows that Col and Ler carry the same pair of *ETC2* alleles specifying, respectively, high and low trichome number (Figure 1C,D), indicating that these two alleles are widespread in the species. The same group who did the current work showed previously that the complete absence of trichomes in several naturally occurring *Arabidopsis* isolates was due to loss-of-function mutations in the coding sequence of *GL1*, which is required for initiation of trichome development [13]. Neither *ETC2* nor *GL1* shows any phenotypes

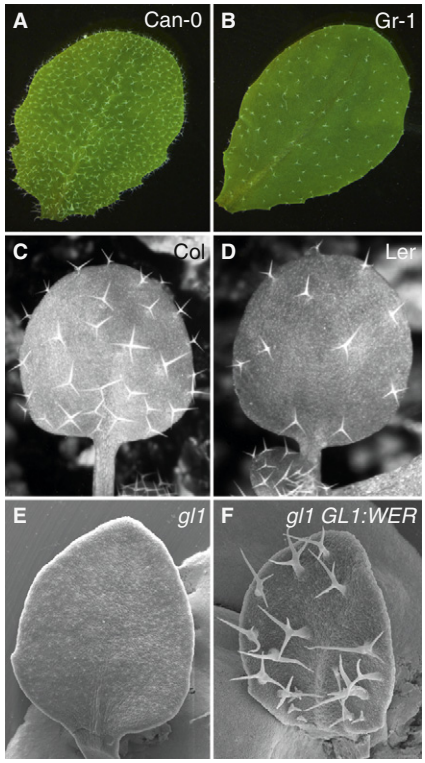

Figure 1. Genetic control of trichome density in *Arabidopsis*.

(A,B) The high and low trichome density lines Can-O and Gr-1 shown by Hilscher *et al.* [8] to carry different alleles of the trichome developmental regulator *ETC2*. (C,D) The high and low trichome density lines Col and Ler, now shown by Hilscher *et al.* [8] to carry the same pair of *ETC2* alleles as Can-0 and Gr-1. (E) *gl1* leaf lacking trichomes. (F) Leaf of a *gl1 GL1cis:WERcoding* plant, showing suppression of the *gl1* trichome phenotype by expression of *WER* from *GL1 cis*-regulatory sequences. Images in (A) and (B) courtesy of Hilscher *et al.* [8]. Images in (E) and (F) courtesy of Myeong Min Lee.

affecting structures other than trichomes, i.e. neither gene is pleiotropic, so this is not a perfect test of the *cis*-regulatory model, but these results support a key role for coding sequence mutations in naturally occurring developmental variation.

But not so fast! A counter example, of sorts, exists within the same *Arabidopsis* regulatory system, though to understand it we need to know something about the control of root hair development in *Arabidopsis*. The root epidermis also contains single-celled hairs, which function in water absorption, but in the root the default state is for each epidermal cell to develop as a root hair cell. A transcription factor complex exists in the root homologous to that which specifies trichomes in the shoot, but this complex functions to specify the non-hair root epidermal cells. Like the trichome transcription complex, the root non-hair complex contains a positively acting MYB transcription factor, WER (a paralog of GL1), a basic helix-loop-helix protein, EGL3 (a paralog of GL3), and TTG, the same WD-repeat protein that functions in trichome development [10]. There are also inhibitory MYBs paralogous to ETC2. As noted above, *gl1* loss-of-function mutants fail to produce trichomes (Figure 1E), and *gl1* mutants do not affect any other aspects of plant development, including root hairs, i.e. the mutants are not pleiotropic. In loss-of-function *wer* mutants, virtually all root epidermal cells develop as root hairs, and no effect is seen on trichomes or other aspects of plant development [14]. WER is 57% identical to GL1 in overall amino acid sequence, and 91% identical within the MYB DNA-binding domain. Yet expression of the *WER* coding region using *GL1 cis*-acting regulatory sequences can completely rescue the *gl1* mutant phenotype and restore fully wild-type trichome development in the leaves (Figure 1F), indicating that all of the functionally significant variation lies in the *cis*-regulatory regions. Of course, gene duplications such as the one leading to *WER* and *GL1* reduce the effects of pleiotropy much as modular *cis*-regulatory regions do.

It seems unlikely that there will be a clear-cut winner in the debate over the role of coding sequence variation vs. *cis*-elements in morphological evolution. To paraphrase Malcolm X's remark about social revolution [15], evolution occurs by any means necessary, and, as noted by Elizabeth Raff above [1], we should expect the molecular record to reveal that evolution uses whatever materials are at hand. Perhaps the more interesting question is to ask without preconception how the selective forces acting on coding sequences and *cis*-regulatory regions differ, how these selective forces are altered by pleiotropy and how these differences affect evolutionary trajectories both within and among species. Versions of the same transcription complex described here are also involved in other aspects of epidermal development, including seed coat development [16] and anthocyanin pigmentation [17], with other examples of both pleiotropy and functional divergence. The control of epidermal development in *Arabidopsis* and its relatives would seem to have much to offer as a laboratory for addressing these questions.

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Evolution: Spatial Scaling of Microbial Interactions

Intraspecific incompatibility in the soil bacterium *Myxococcus xanthus* demonstrates that the social life of microbes is antagonistic on local and global scales. Antagonistic interactions and non-self recognition are likely to promote microbial diversity in local populations.

Rachel J. Whitaker

Although it was once believed that microbes are cosmopolitan [1], it is now clear that at least some microbes evolve in geographically isolated populations [2,3]. When examined with molecular markers many microbial species, including those capable of forming resistant spores that disperse easily, exist as local sub-populations separated by migration barriers [4,5]. Even viruses and other mobile genetic elements exhibit biogeographic