Dispatches

Evolutionary Genetics: How Flies Get Naked

Researchers studying evolution of 'naked' (hairless) larval cuticle in *Drosophila sechellia* have discovered surprising complexity in the pattern of *cis*-regulatory differences that differentiate this species from its 'hairy' ancestors.

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With the advances in DNA sequencing technology, a comprehensive catalog of genetic differences between species can now be readily obtained. Identifying the subset of differences that affect a specific trait, however, remains a considerable challenge. Consequently, many fundamental questions about the genetic basis of divergent traits remain unresolved: Is divergence caused by one, a few or many genes? Which genes are affected? Which changes within these genes cause phenotypic divergence and how do they impact biological systems? Each trait will have unique answers to this set of questions, but should we expect commonalities in evolutionary genetic mechanisms?

Different traits are controlled by different sets of genes, yet many properties of developmental systems are shared among multi-cellular organisms. This suggests that common features of evolutionary mechanisms will emerge [1]. One such feature already emerging is that changes in gene regulation often contribute to phenotypic divergence. This feature of evolutionary divergence is rationalized by the ability of mutations affecting tissue-specific, modular, *cis*-regulatory elements to have fewer negative 'side-effects' than mutations in protein coding sequences [2]. In the last decade, case studies demonstrating differences in gene expression that correlate with divergent traits have accumulated rapidly.

Although most comparative expression studies stop after observing a phenotypic correlation, a handful of studies have gone a step further toward establishing a causative role of divergent gene expression by recreating species-specific expression patterns in model organisms (for example [3–6]). In some cases, these experiments produce morphological changes similar to the divergent phenotypes. For example, wider beaks in Darwin's finches were found to correlate with increased expression of the *BMP4* gene, and manipulating expression of this gene in chicken embryos produced changes in beak shape that mirrored those seen in the finches [3]. In other cases, such as for pigmentation evolution in *Drosophila*, changing expression of a single gene is insufficient to produce the divergent phenotype [7], at least in some genetic backgrounds [6]. In these latter cases, observed expression differences may be necessary but not sufficient for the divergent phenotype. These studies provide compelling arguments for the importance of regulatory divergence in evolution, but they do not identify the mutations that are the ultimate genetic cause of phenotypic differences.

Genetic mapping, combined with expression analysis, is the best way to prove beyond a reasonable doubt that regulatory mutations underlie phenotypic divergence (for example [8]). A new study by McGregor *et al.* [9] has achieved this level of resolution using a combination of fine-scale genetic mapping and transgenic analysis. They have uncovered a previously unseen complexity in the structure of *cis*-regulatory divergence, and shown that, despite many potential ways to alter a phenotype, some genes are more likely than others to be the cause of morphological evolution.

McGregor *et al.* [9] examine the divergence of larval cuticle phenotypes among *Drosophila* species. As shown in Figure 1A, *Drosophila* larvae contain hair-like structures called trichomes on their body cuticle. During the first larval instar, all members of the *Drosophila melanogaster* group except *D. sechellia* have a lawn of trichomes on the dorsal side of each abdominal segment (Figure 1B). In *D. sechellia*, these trichomes fail to form, leaving only naked cuticle [10]. Four members of the *Drosophila virilis* group, which diverged from the *melanogaster* group approximately 60 million years ago, have also evolved naked larval cuticle [11]. This naked phenotype is the derived state in both groups, indicating that the genetic changes responsible for these phenotypes are independent, and that evolution of naked cuticle in the *melanogaster* and *virilis* groups should not have a similar genetic basis that is due to shared ancestry.

Interspecific crosses of *D. sechellia* with *D. melanogaster* and *D. simulans*, both 'hairy' species, showed that only one region of the genome, located on the X chromosome, is responsible for suppressing the development of trichomes [10]. Taking advantage of the available genetic resources in *D. melanogaster*, deficiency mapping and complementation testing were used to show that the *shavenbaby* (*svb*) gene is responsible for the interspecific difference in trichome pattern. Interspecific genetic mapping was also attempted in the *virilis* group, but incompatibilities among species prevented the formation of hybrids in most cases [11]. Analysis of the few hybrids that were obtained showed phenotypes and genotypes that were consistent with

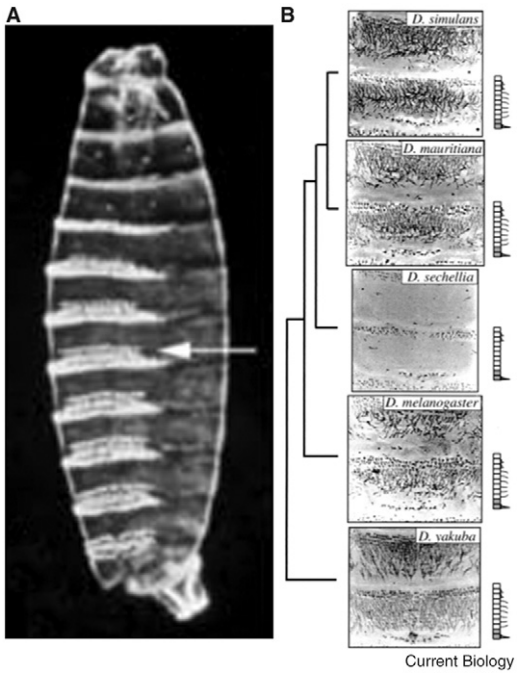

Figure 1. Larval trichome patterns in the *D. melanogaster* group.

(A) *D. melanogaster* displays broad denticle belts containing many trichomes on the dorsal surface of each abdominal segment (arrow). (B) The lawn of larval trichomes present in all other members of the *melanogaster* group is absent in *D. sechellia*. (A modified from [17], and B reproduced with permission from [10]).

species-specific alleles of the *svb* gene also determining the larval phenotype in the *virilis* group.

Many genes affect the development of larval trichomes, so why has *svb* been used to evolve naked cuticle multiple times independently? One potential answer arises from the location of the *svb* gene in the developmental pathway controlling trichome development. Signal transduction pathways, which are used for development of many structures in the fly, affect larval trichome formation by regulating expression of the *svb* gene [12]. The *svb* gene in turn controls the production of trichomes by regulating expression of the structural genes that actually construct the cell type [13]. Mutations are expected to occur at similar rates in all parts of this pathway; however, changes affecting regulation of *svb* may be the only ones specific enough for trichome development that they avoid disrupting other body parts, yet also powerful enough to dictate the presence or absence of trichomes. Genes controlling development of a specific cell type that have minimal effects on other traits may be prime targets for evolutionary change in general. If so, elucidating the structure of developmental pathways will help predict the most likely genetic targets for evolutionary change.

As the name *shavenbaby* suggests, mutations in this gene inhibit trichome formation in young flies. However, the *svb* locus is also required for the proper function of the female germ-line. Distinct *cis*-regulatory regions and alternative splicing of transcripts separate these two different functions [14]. Comparisons of *svb* transcripts among species in the *melanogaster* and *virilis* groups revealed differences in *svb* expression that correlate specifically with the larval cuticle phenotypes [10,11]. That is, expression is conserved except in the polymorphic ('naked'/'hairy') regions. These changes in a subset of the expression pattern suggest that divergence of a tissue-specific *cis*-regulatory region is responsible for divergent expression.

To identify the *cis*-regulatory sequences of *svb* controlling expression in the first instar larval cuticle, regions of non-coding DNA upstream of and within the *svb* gene were fused to a heterologous reporter gene and introduced into *D. melanogaster* [9]. Surprisingly, three separate *cis*-regulatory regions were found to drive expression in distinct, but overlapping patterns within the region of interest. Activities of homologous regions from *D. sechellia* were examined, and changes decreasing gene expression were found to have occurred in all three *cis*-regulatory sequences. Fine-scale genetic mapping within the upstream region of *svb* showed distinct phenotypic consequences of each change; divergence of each *cis*-regulatory region eliminates a different subset of trichomes. The complete loss of larval trichomes in *D. sechellia* requires all three divergent *cis*-regulatory regions of the *svb* gene. This finding demonstrates how an allele with a large phenotypic effect can be constructed by combining individual changes with smaller effects. It also nicely illustrates the power of using genetic analysis to investigate divergence between closely related (and interfertile) species. Further investigation into the nature of divergence within each *cis*-regulatory region of *svb* will provide an even greater understanding of the molecular genetic mechanisms underlying phenotypic evolution.

Finally, this study [9] allows the adaptive nature of evolutionary changes in larval trichome patterning to be addressed for the first time. In comparison to other systems used to investigate evolutionary genetic mechanisms — such as *Drosophila* wing spots [7,15], stickleback body armor [16], morphology of domesticated corn [8] — the ecological relevance of larval trichomes is less clear. Nonetheless, the discovery of multiple changes fixed in the *D. sechellia svb* gene argues strongly that selection is the driving force behind the evolution of naked cuticle. While it is plausible to think that neutral processes such as genetic drift could have fixed one mutation within a gene, the probability that multiple changes in the same gene, all with similar effects, were fixed by chance alone is miniscule.

References

1. Davidson, E.H., and Erwin, D.H. (2006). Gene regulatory networks and the evolution of animal body plans. Science *311*, 796–800.

2. Wray, G. (2007). The evolutionary significance of cis-regulatory mutations. Nat. Rev. Genet. *8*, 206–216.
3. Abzhanov, A., Protas, M., Grant, B., Grant, P., and Tabin, C. (2004). Bmp4 and morphological variation of beaks in Darwin's finches. Science *305*, 1462–1465.
4. Sliwinski, M., White, M., Maizel, A., Weigel, D., and Baum, D. (2006). Evolutionary divergence of LFY function in the mustards Arabidopsis thaliana and Leavenworthia crassa. Plant Mol. Biol. *62*, 279–289.
5. Weatherbee, S., Behringer, R., Rasweiler, J., and Niswander, L. (2006). Interdigital webbing retention in bat wings illustrates genetic changes underlying amniote limb diversification. Proc. Natl. Acad. Sci. USA *103*, 15103–15107.
6. Wittkopp, P., Vaccaro, K., and Carroll, S. (2002). Evolution of yellow gene regulation and pigmentation in Drosophila. Curr. Biol. *12*, 1547–1556.
7. Gompel, N., Prud'homme, B., Wittkopp, P., Kassner, V., and Carroll, S. (2005). Chance caught on the wing: cis-regulatory evolution and the origin of pigment patterns in Drosophila. Nature *433*, 481–487.
8. Clark, R., Wagler, T., Quijada, P., and Doebley, J. (2006). A distant upstream enhancer at the maize domestication gene tb1 has pleiotropic effects on plant and inflorescent architecture. Nat. Genet. *38*, 594–597.
9. McGregor, A., Orgogozo, V., Delon, I., Zanet, J., Srinivasan, D., Payre, F., and Stern, D. (2007). Morphological evolution through multiple cis-regulatory mutations at a single gene. Nature *448*, 587–590.
10. Sucena, E., and Stern, D. (2000). Divergence of larval morphology between Drosophila sechellia and its sibling species caused by cis-regulatory evolution of ovo/shaven-baby. Proc. Natl. Acad. Sci. USA *97*, 4530–4534.
11. Sucena, E., Delon, I., Jones, I., Payre, F., and Stern, D. (2003). Regulatory evolution of shavenbaby/ovo underlies multiple cases of morphological parallelism. Nature *424*, 935–938.
12. Payre, F., Vincent, A., and Carreno, S. (1999). ovo/svb integrates Wingless and DER pathways to control epidermis differentiation. Nature *400*, 271–275.
13. Chanut-Delalande, H., Fernandes, I., Roch, F., Payre, F., and Plaza, S. (2006). Shavenbaby couples patterning to epidermal cell shape control. PLoS Biol. *4*, e290.
14. Salles, C., Mével-Ninio, M., Vincent, A., and Payre, F. (2002). A germline-specific splicing generates an extended ovo protein isoform required for Drosophila oogenesis. Dev. Biol. *246*, 366–376.
15. Prud'homme, B., Gompel, N., Rokas, A., Kassner, V.A., Williams, T.M., Yeh, S.D., True, J.R., and Carroll, S.B. (2006). Repeated morphological evolution through cis-regulatory changes in a pleiotropic gene. Nature *440*, 1050–1053.
16. Colosimo, P., Hosemann, K., Balabhadra, S., Villarreal, G., Dickson, M., Grimwood, J., Schmutz, J., Myers, R., Schluter, D., and Kingsley, D. (2005). Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. Science *307*, 1928–1933.
17. Wehrli, M., Dougan, S.T., Caldwell, K., O'Keefe, L., Schwartz, S., Vaizel-Ohayon, D., Schejter, E., Tomlinson, A., and DiNardo, S. (2000). arrow encodes an LDL-receptor-related protein essential for Wingless signalling. Nature *407*, 527–530.

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Brain Evolution: When Is a Group Not a Group?

In testing the 'social brain hypothesis' with comparative data, most research has used group size as an index of cognitive challenge. Recent work suggests that this measure is too crude to apply to a wide range of species, and biologists may need to develop other ways of extending these analyses.

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The overall size of the brain and the relative enlargement of its component parts vary widely across species. Part of this variation can be accounted for by allometric changes with body size, and part by correlated increases among many brain parts linked by developmental constraints; but much remains to be explained as adaptive variation. In pinning down the sources of this variation, the usual approach is to seek correlations with environmental challenges faced by different species. To do that, it is necessary to index the possible challenges in a robust and simple way, so that a large number of species can be included in the analysis. In this way, social complexity, a key cognitive challenge according to the Machiavellian intelligence hypothesis [1], has usually been reduced simply to group size. Admittedly crude, this at least captures the idea that information-processing demands should, on average, increase with the number of social relationships an individual has to deal with [2].

Anthropoid primates are a paradigm case where this is true [3], and species with larger neocortex do indeed live in larger groups [4]. Moreover, the same principle seems to apply in other taxa: chiropteran bat species that show stable social groups have larger neocortex than those that do not [5], and the most social cetacean species are also those with the largest brains [6]. There is reason, therefore, to think that the selective effect on brain enlargement from social complexity — as measured by group size — is a general one at least in mammals. Recently, Shultz and Dunbar [7,8] have increased the level of sophistication in such analyses by including another measure of social complexity: a species' social organization. They found powerful effects on brain size in all taxa analysed.

Firstly for even-toed ungulates [7], a group with a convenient lack of dietary complexity, and now also for carnivores and birds [8], Shultz and Dunbar report that, whereas group size has weak and often inconsistent effects on relative brain size, a species' social system is closely related to the size of its brain. Intriguingly, in these non-primate species they found monogamy to be more closely associated with brain enlargement than is group size; it was not the highly competitive, multi-male mating systems which were particularly linked to brain enlargement, but rather having harems and especially pair-bonding. In contrast, among primates, multi-male mating systems (and larger group sizes)