Insect evolution: Redesigning the fruitfly

Greg Gibson

Homeotic mutations in *Drosophila* can result in dramatic phenotypes that suggest the possibility for rapid morphological evolution, but dissection of the genetic pathway downstream of *Ultrabithorax* is beginning to reveal how wing morphology may have evolved by more gradual transformations.

Address: Department of Genetics, Gardner Hall, North Carolina State University, Raleigh, North Carolina 27695-7614, USA.
E-mail: ggibson@unity.ncsu.edu

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Among the more spectacular achievements of molecular biologists in the mid-1980s was the demonstration that hundreds of millions of years of evolution could be undone in a couple of weeks by the clever exploitation of techniques for generating 'transgenic' organisms. Schneuwly et al. [1], for example, redesigned the antennae of the fruitfly *Drosophila melanogaster* into a pair of legs, simply by using a heat shock promoter to deliver a pulse or two of Antennapedia protein to the antennal imaginal disc at the appropriate time in development. When this result was quickly replicated using the mouse, rather than *Drosophila*, version of the gene [2], the deep similarities in the mechanisms by which the body plan is established throughout the animal kingdom were clear to all. As well as capturing the imagination, these experiments signaled the beginning of a new era in the study of development and evolution, as the goal of understanding morphological divergence at the level of changes in gene expression came into view.

One of the first things we learn in primary school natural history classes is that butterflies have four wings, whereas regular (dipteran) flies have just a single pair of wings. In some places, students are also taught about evolution, and may learn that the dipterans are a derived state, in the sense that the ancestors of fruit flies and house flies had four wings, but that 200 million years or so ago the hind pair was reduced to a balancing organ called a 'haltere'. Some of us are later taught the additional fact that there are a series of 'homeotic' mutations in *Drosophila* that change the identities of body parts, one of which — *Ultrabithorax*, *Ubx* for short — reverses evolution by turning the halteres back into wings. It would seem, then, that understanding how homeotic genes work — which in a nutshell is by regulating the transcription of a variety of target genes — provides a key to understanding major evolutionary innovations. The particularly daunting problem is to understand how major transitions between stable morphologies occur, whether through revolutionary mutations or the gradual accretion of segregating variation.

A research program for getting at this problem has emerged for a number of systems, and can be boiled down to some combination of the following practical steps. First, document the number and identity of target genes that are regulated by master control genes in a genetically tractable organism such as *Drosophila*. Second, ascertain how many and which of these targets are also targets in other species, including divergent and closely related ones. Third, establish the nature of the DNA changes that have accompanied changes in gene expression between species. Fourth, test the phenotypic consequences of reconstructing particular evolutionary changes in target gene expression. And fifth, compare these changes with segregating variation to try to put together a reasonable model of the rate and mode of evolutionary divergence. This program is beginning to bear fruit and is providing reassuring support for those who favour a 'gradualist' view of evolution.

A haltere is not a hindwing is not a forewing

Progress on the second of these aims is reported by Weatherbee et al. in this issue of *Current Biology* [3]. A few years ago, this group demonstrated [4] that the qualitative difference between halteres in fruitflies and hindwings in *Precis coenia* butterflies must be due to changes in what the Ubx protein does rather than where it is expressed, as the protein is found uniformly throughout both sets of imaginal discs (but not in the forewings). Last year [5], they showed in a seminal paper, which took advantage of recent progress in the understanding of the genetic patterning of wings, that altered gene regulation occurs at several levels in the downstream hierarchy of target genes [6]. Their new work [3] clearly demonstrates that there are abundant differences in *Ubx* target gene regulation between the haltere and hindwing imaginal discs, as illustrated in Figure 1. Thus, despite the extremely high conservation of homeotic protein sequences across taxa, there is considerable freedom in the evolution of what they regulate.

The most obvious difference between halteres and wings is that the former are much reduced in size, particularly on the posterior side. This feature corresponds well with the suppression in haltere imaginal discs of the transcription of two key regulatory genes, *wingless*, which encodes a signaling molecule that organizes the dorsoventral axis and is ultimately necessary for cell proliferation in wing discs, and *SRF* (also known as *blistered*), which is required for development of cells between the veins. But both of these genes are expressed in *P. coenia* hindwings, just as they are...
in the forewings. Another gene, part of the Achaete-scute complex that is required for differentiation of bristle cells along the wing margin, is also down-regulated in haltere imaginal discs but not in hindwing discs. These observations indicate that, between fruitflies and butterflies, changes in target gene specificity have occurred at multiple levels in the regulatory hierarchy downstream of Ubx.

Weatherbee et al. [3] also report examples of differences in gene expression that presumably contribute to the marked morphological differences between butterfly forewings and hindwings, as well as between the butterfly forewings and fruitfly wings [5]. For example, two novel stripes of wingless expression that appear to mark the location of colored bands of scales on the forewings are not seen in hindwing imaginal discs, and have no counterpart in Drosophila. It is likely that an understanding of the molecular basis of wing scales and hindwings, which differ instead in the degree of activation of genes that help to pattern the scales, including Distalless in the posterior eyespot (red) and wingless in a novel domain consisting of two proximal stripes of cells (light blue).

Genetic variation in pathways and networks
What do these results imply about the nature of the evolutionary pathways leading to the dramatic morphological transitions that we observe when we compare species? This remains very much a black box, but we can at least begin to model possible evolutionary scenarios. As illustrated in Figure 2, homeotic genes must regulate a number of different processes to orchestrate appendage development. Intuitively, the structure or ‘architecture’ of a regulatory pathway will have a large effect on the manner in which it can evolve. This proposition is yet to be incorporated into mathematical population or quantitative genetic theory, and is largely a matter of prejudice among experimentalists.

There is a tendency, perhaps borrowing from studies of metabolism and embryonic segmentation, to conceive of developmental pathways as being very sequential, with each level of gene activity handing on responsibility to the next level. For example, homeotic genes could regulate local arbiters of positional information, such as wingless, which in turn control proliferation or differentiation through genes like SRF and the Achaete-scute complex. But there is much evidence from clonal analysis that genes like Ubx and Antennapedia retain local effects on cell differentiation that are independent of the global patterning of the imaginal discs, and there is accumulating evidence that they directly control the transcription of ‘structural’ as well
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Conventional wisdom has it that genes at the top of linear hierarchies are likely to be 'entrenched' [8]. This means that any small change in activity will cascade into a major change in morphology that would almost always be deleterious, and hence that such genes are unlikely to contribute to evolution. Some authors nevertheless see in this a mechanism for the creation of 'hopeful monsters'. By contrast, the more interactions there are in a pathway, the more likely it is to evolve toward a stable state, in which modification of the activity of one gene is buffered by the whole [9]. In this case, the paradox emerges that, despite phenotypic uniformity, the underlying genetic architecture may remain quite labile and in a state of perpetual flux. It is thus interesting to ask at what levels genetic variation affecting appendage development occurs.

That variation in the activity of Ubx itself can contribute to interspecific divergence has been shown in a neat set of experiments by David Stern [10]. The number of 'trichomes', or fine hairs, on the posterior side of the femur on the second leg of a fly is to some extent species specific. Modulation of the dosage of wild-type Ubx activity affects the extent of trichome coverage in D. melanogaster and mutant clones lacking Ubx expression gain trichomes, indicating that Ubx protein represses trichome development. By inducing novel Ubx mutations in highly inbred lines of D. melanogaster and D. simulans, and then measuring the extent of trichome coverage in hybrid flies that contained just one copy of Ubx derived from either species, Stern demonstrated further that the difference between these two species is at least in part due to divergence in the Ubx gene. As there were no changes in Ubx protein sequence between the species, the affect is attributable to divergence in the extensive cis-regulatory enhancer sequences that govern Ubx expression.

The molecular nature of interspecific divergence in regulatory function has been explored in more detail in the case of the so-called 'stripe-2 enhancer' of the segmentation gene even-skipped. This enhancer is sufficient to drive even-skipped expression in a narrow stripe of blastoderm cells, and acts by binding with a range of affinities to several well known transcription factors of the gap gene class. Ludwig et al. [11] have demonstrated that the precise number and location of binding sites for particular transcription factors in the even-skipped stripe-2 enhancer differs between species, but remarkably the different enhancers are still able to drive expression of a reporter gene in the correct pattern (though probably at different quantitative levels). Consequently, the loss of one binding site can be compensated for by the gain of another, and there is genetic turnover that is apparently without great consequence for the organism.

These two sets of results both suggest that there should also be genetic variation within a species relating to function of homeotic and other classes of regulatory gene. At a superficial level, there does not appear to be such variation: all fruitflies have two and only two wings. But we have shown [12] that different genetic backgrounds have highly heritable effects on separable homeotic phenotypes, such as the size of the enlarged haltere in Ubx heterozygotes or the number of bristles along the margin of the haltere, as well as the degree of antenna-to-leg transformation in Antennapedia gain-of-function mutants. Furthermore, single quantitative trait loci that have no observable effect on wild-type flies can have a very large effect when the genetic system is perturbed (see for example [13]). There is thus genetic variance segregating in natural populations that can potentially affect the activity of genes acting at various levels of appendage development, even though the general morphology of legs and wings is invariant.

Understanding the relationship between this variation, interspecific divergence and the qualitatively distinct appendages that develop in divergent taxa remains a serious challenge. But this challenge will have to be met before developmental studies of evolution can be effectively integrated with the neo-darwinian synthesis. The new results imply that divergence in genetic pathways can occur at a variety of levels, from the regulation of homeotic gene activity to the fine-tuning of cell differentiation.
Once changes in specific target genes have been identified, we will be in a position to address the sequence of changes that led to morphological divergence, and eventually the roles of selection, drift and historical contingency in macroevolutionary change.

References