Evolution: **A complement for evolutionary genetics**

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Developmental geneticists' contribution to the study of the evolution of morphological divergence has proceeded along two lines: comparative analysis of gene expression and quantitative genetics. Recent studies highlight how complementation tests between species can bridge the gap between these approaches.

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Alongside mutation, a geneticist's best friend is the complementation test. In the absence of epistasis, failure to complement is a sign of allelism, an indication that two or more mutations that reduce or eliminate function affect the same gene. Even in the presence of genetic interactions, complementation can hint at the structure of a genetic pathway. Classically implemented, complementation tests require genetic crosses and hence are restricted to studies within a species. With a little imagination, though, the basic idea can be extended to dissection of the genetic basis of evolutionary divergence.

Many of the spectacular studies of comparative evolution and development over the past decade [1] have relied upon what might uncharitably be referred to as fishing expeditions. Productive and pleasing expeditions to be sure, but nevertheless ones based on comparisons of expression patterns of a series of genes chosen simply because they are already known to be involved in the development of the homologous trait in a model reference organism. This strategy is terrific for comparisons at higher taxonomic levels, as we know a lot about the developmental genetics of body plans. When it comes to the more subtle changes that distinguish closely related species, though, with the possible exception of bristle patterning, we do not have enough knowledge to even guess where to begin a study. This is where complementation approaches can show their worth.

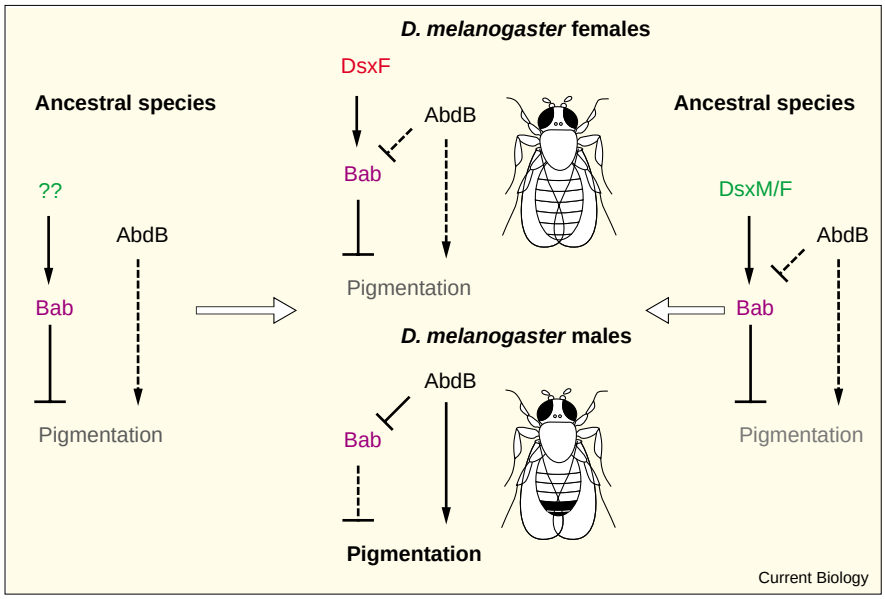
The basic idea of a complementation test is to ask whether the combination of two different mutations produces the same (or a similar) phenotype to that produced by homozygosity for one of the mutations. Such direct tests cannot be performed to compare non-interbreeding related species, but it is feasible at least to look for mutations in one species that produce the same phenotype as is observed in wild-type members of the related species. If the mutation results in reversion to the ancestral state, it is called an atavistic mutation, and the inference is made that the gene identified lies on the genetic pathway in which evolution has occurred. Importantly, this is not a test of allelism, so it is not correct to conclude that the gene contributed to the evolution. A famous example is the four-winged *Ultrabithorax* phenotype in flies, which implicates the homeotic pathway, but not the *Ubx* gene itself, in the transition to the two-winged dipteran body plan [2].

As a first step toward characterizing the molecular basis for a sex-specific difference in pigmentation in the *melanogaster* species group of *Drosophila*, Kopp *et al.* [3] have looked for atavistic mutations that lead to loss of pigmentation on the fifth and sixth abdominal segments of males. Mutation of the well-known homeotic gene *AbdB* had this effect, while mutations of two other genes, *doublesex* (*dsx*) and *bric-a-brac* (*bab*), had the opposite effect of inducing ectopic pigmentation in the same segments of females, which are normally non-pigmented in most *Drosophila* species. The authors then observed that the *bab* effect is suppressed by heterozygosity for *AbdB*, and this instance of intergenic complementation hints that the two genes may lie in the same pathway.

After a series of further manipulations with transgenes, as well as analysis of the expression of the Bab protein, Kopp *et al.* [3] concluded that *bab* is repressed by AbdB in both sexes, but that this effect is overridden by the female version of the Dsx protein in females (Figure 1). Consequently, in *melanogaster* group males, Bab protein is absent in A5 and A6, so it is not available to antagonize the function of AbdB in promoting pigmentation. As *AbdB* and *dsx* perform so many crucial functions in patterning of the abdomen, expression of these genes is tightly constrained, so the authors developed the testable hypothesis that evolution of *cis*-regulatory elements of *bab* that respond to AbdB and Dsx has given rise to the sex-specific pigmentation pattern.

Interspecific genetic dissection has been taken a step further by Sucena and Stern [4] in their demonstration that *cis*-regulatory evolution at the *shaven baby* locus is solely responsible for the loss of hairs on the dorsal larval cuticle in *D. sechellia*. This condition is unique to the *melanogaster* group of species, and was shown by interspecific backcrosses to be attributable to a sex-linked dominant Mendelian locus. The authors proceeded to cross females carrying one of a panel of deficiencies that collectively cover 80% of the *D. melanogaster* X chromosome, to

Figure 1

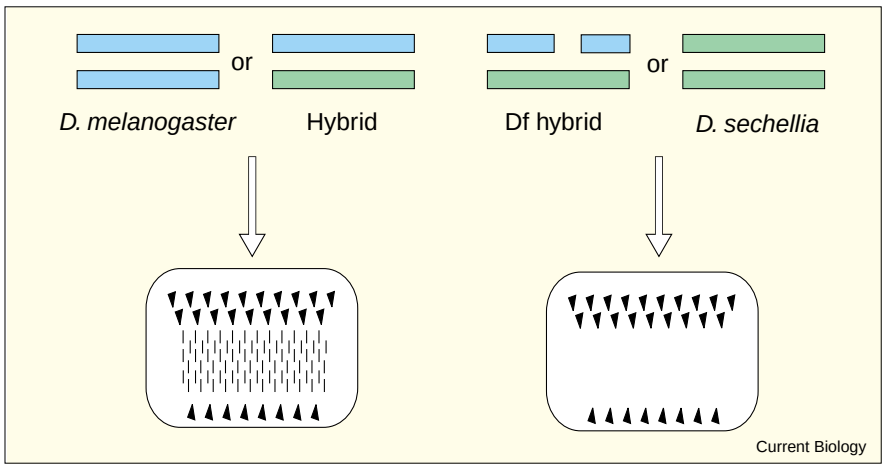


Evolution of sex-specific abdominal pigmentation in *D. melanogaster*. In ancestral *Drosophila* species, pigmentation in the abdomens of both sexes is repressed by Bab, expression of which is induced by unknown factors (left). Kopp *et al.* [3] show that, at least in the *melanogaster* group, *bab* is repressed in males by AbdB (center, bottom), but that this effect is overcome in females as a result of activation by the female version of the Dsx protein (center, top). As Bab also regulates other aspects of sex-specific abdominal morphology, Dsx proteins may also regulate *bab* expression in ancestral species (right), in which case the loss of expression of *bab* in *melanogaster* group males would have evolved by loss of responsiveness to just the male version of the Dsx protein.

D. sechellia males. They observed that several deficiencies in the 4DE region failed to complement the *D. sechellia* allele, resulting in female larvae with naked dorsal cuticle (Figure 2). Furthermore, loss-of-function mutations of the aptly named *shaven baby* gene had the same effect, while *in situ* hybridizations confirmed that, in *D. sechellia*, expression of the gene is missing from just that portion of the presumptive cuticle that is affected.

These studies are also of interest in light of their implications for the role of so-called macromutations in evolution. The *shaven baby* story is particularly clear cut in implicating a single mutation as the cause for a clean morphological difference, though it remains possible that this locus alone has picked up multiple mutations each of small effect. It is much less clear that *bric-a-brac* is solely responsible for the evolution of the pigmentation sexual dimorphism, though Kopp *et al.* [3] also cite data mapping a major-effect modifier of female pigmentation segregating within *D. melanogaster* to the vicinity of this locus [5]. These studies add to a growing body of evidence from quantitative trait loci (QTL) mapping that mutations of large effect can contribute to evolutionary divergence, as well as to segregating variation.

Figure 2



Failure to complement identifies *shaven baby* as the gene responsible for the evolution of naked cuticle in *D. sechellia* [4]. As hybrid females derived from the cross of *D. melanogaster* females to *D. sechellia* males resemble *D. melanogaster* in having dorsal larval cuticle covered in six rows of hairs per segment (left), the naked cuticle phenotype in *D. sechellia* is recessive to the ancestral condition. In hybrids carrying a chromosomal deficiency that includes the *shaven baby* locus, the *D. sechellia* phenotype is produced (right), indicating that a loss-of-function mutation contained within the deficiency was fixed in *D. sechellia*. Three rows of more robust denticles per segment are unaffected. A similar design was used by Takano-Shimizu [9] in his study of bristle anomalies in hybrids between *D. melanogaster* and *D. simulans* or *D. mauritiana*.

On the face of it, such data flies in the face of the long accepted tenet of the modern synthesis, that only mutations of very small effect contribute to evolutionary change under positive selection. This notion traces to R.A. Fisher's persuasive argument [6] that, in the presence of pleiotropy, the likelihood that a mutation of large effect is adaptive is so small as to be negligible. Subsequent mathematical treatments have softened this view, notably one based on Kimura's reflection [7] that, as those few major-effect variants that happen to be adaptive have a much improved probability of fixation, factors of at least intermediate effect should be expected to distinguish species. Most recently, Allen Orr has argued [8] that any walk to a new fitness optimum will entail multiple substitutions, at least one of which is likely to have a considerably larger effect than the others.

It would be premature to conclude that the new data and theory should lead us to embrace macromutations as the stuff of evolution. For one thing, there is undoubtedly an ascertainment bias in the study of qualitative transitions such as loss of pigmentation or baldness. As both groups point out [3,4], there are other subtle, quantitative changes in segment and hair morphology associated with their traits, which will be much more difficult to dissect. Further, the mathematical theory of adaptation assumes movement toward an intermediate optimum, whereas complete loss of something is movement toward an extreme. Loss is also a distinct process from the emergence of novel features by gain of function.

Interspecific complementation tests carried out by Toshi Takano-Shimizu [9] are also relevant to assessing the prevalence of macromutational effects. He has shown that a series of deficiencies of the *D. melanogaster* X chromosome fail to complement the *D. simulans* X chromosome in hybrid females, resulting in loss of a considerable number of bristles from the adult notum — despite the fact that both pure species have the same wild-type pattern of bristles! The implication is that substitutions with effects potentially as large as those observed at the *shaven baby* locus are being fixed between species on a regular basis, but their effects are masked by prior or accompanying substitutions at other epistatically interacting loci, just as Dobzhansky envisaged [10]. These substitutions also contribute to the evolution of hybrid incompatibility [11,12] and complementation testing is emerging as a vital tool in the dissection of this phenomenon, which lies at the heart of speciation. A truly robust picture of the relationship between interspecific and intraspecific variation will be built upon these studies, once we have the power to resolve morphological effects down to the nucleotide, and to test for the extent of interactions among individual substitutions.

In the meantime, the new results also bring the study of homoplasy within the realm of developmental genetics. Features such as abdominal pigmentation and wing venation are tremendous markers of species identity, but of debatable phylogenetic utility as they appear to evolve repeatedly. Once mutations such as *bric-a-brac* and *blistered* — which results in an atavistic wing venation phenotype [13] — have been placed in the pathway responsible for homoplasic features, it becomes feasible to test whether these genes have repeatedly played a role in the evolution of the trait. Kopp *et al.* [3] provide some evidence that pigmentation has evolved independently of a change in *bab* expression in a subset of species outside the *melanogaster* group, and they also cite examples where the changes have been restricted to the sixth abdominal segment. It will be fascinating to see whether concerted evolution has occurred at the level of the single gene. Dissection of the genetics of homoplasy will undoubtedly also have consequences for phylogeny reconstruction, the inference of homology, and evaluation of the impact of developmental constraints on the evolutionary process.

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