

Insect evolution: **How the fruit fly changed (some of) its spots**

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Recent results show that the establishment of wing melanization patterns in *Drosophila* depends on the veins. While several genes have been shown to play a role in melanization, changes in a single gene – the one encoding tyrosine hydroxylase – are sufficient to generate novel pigmentation patterns.

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Animals display a wide variety of markings and patterns, from the stripes of zebras to the spots on the wings of butterflies. The significance and genesis of these spots and stripes have long been the subject of speculation. Evolutionary biologists have suggested many possible reasons why species exhibit such patterns, including mimicry, mate recognition and aposematism (where the pattern acts as a signal to potential predators that the carrier is distasteful or poisonous). Similarly, theoretical biologists, starting with Turing [1] and more recently Goodwin [2] and Stewart [3], have postulated reaction–diffusion models to explain how the pigmentation patterns are generated. While these models can be highly predictive of the patterns observed in vertebrates, their potential role in insect pigmentation remains unclear.

As wing pigmentation patterns are often involved in important aspects of insect life history, and because wing markings are used as diagnostics for species designations and as characters in phylogenetic analysis, understanding of the molecular basis of this phenotype is of considerable interest. The biochemical pathways involved in melanization, and the mechanisms by which the pigmentation prepattern is established, have been the subject of extensive study in the model organism *Drosophila melanogaster*. As recently reported in *Current Biology*, True *et al.* [4] have made significant progress in deciphering the molecular genetic basis of wing pigmentation patterning in *D. melanogaster*.

Painting the wings of flies

True *et al.* [4] have shown that the establishment of wing pigmentation patterns in *Drosophila* depends on two major factors: first, interactions affecting the genes that encode the enzymes of melanization; and second, the hemolymph supply system provided by the wing veins. The first factor determines the pattern of production of the precursors that eventually make melanin. The second component of

this system is the transport system that deposits the precursors in a prepattern in the wing. True *et al.* [4] suggest that these findings may also explain pigmentation patterns in the wings of a wide range of other insect species.

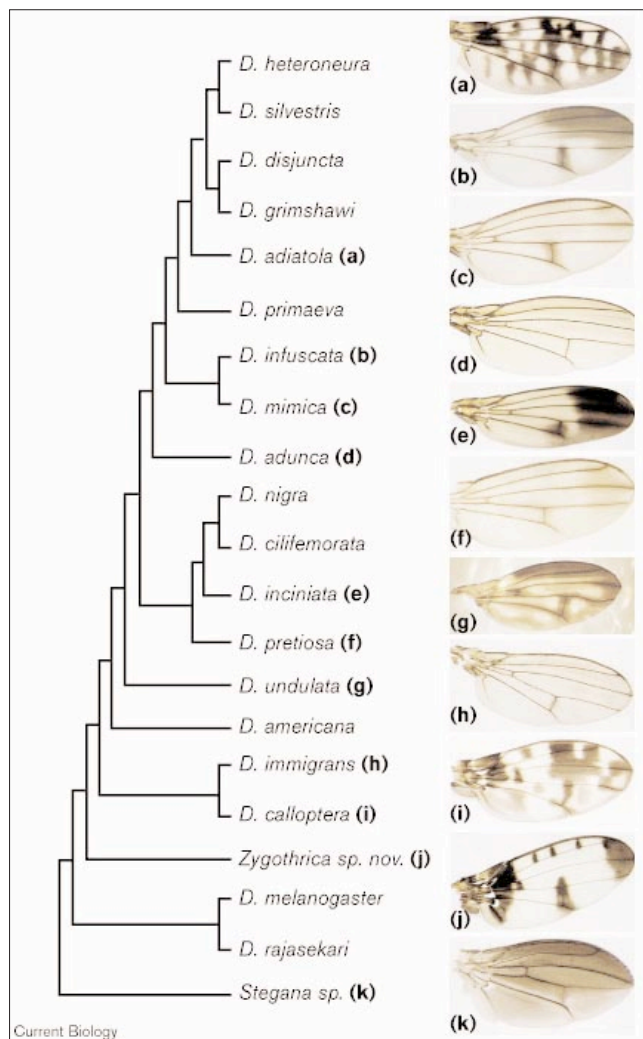
The astonishing degree of pigmentation pattern variability in wings of the Drosophilidae (Figure 1) makes this family an obvious choice for studying how pigmentation patterns change during evolution, using as a starting point the molecular genetic insights gained from work on *D. melanogaster*. Species in this family have wings which range from completely clear to almost entirely black. Between these extremes, species can have wings that are either slightly or heavily tinged with brown along the crossveins and apex, have complex patterns of pigmentation, or possess any combination of these characters. Most members of the Hawaiian picture-wing clade have highly complex patterns on their wings. Several mutants in this group seem to be phenocopies (*sensu* Basile and Stebbins [5]) of some *D. melanogaster* mutants, making it likely that it will be possible to extend some of the genetic findings in *D. melanogaster* across a broader range of species.

The biochemical palette

The biochemical pathway for the production of melanin has been extensively studied [6]. Previous studies indicate that tyrosine hydroxylase and dopa decarboxylase are key participants in the biosynthesis of melanin, and obvious candidates for further study. Using mitotic clonal analyses, True *et al.* [4] showed that both dopa decarboxylase and tyrosine hydroxylase are necessary for the formation of melanin in the abdomen of *D. melanogaster*. But the absence of wing melanin patterns in this species makes it difficult to directly test whether these loci are involved in wing pattern formation. To investigate further the role of tyrosine hydroxylase and dopa decarboxylase in wing pigmentation, True *et al.* [4] expressed their genes — *TH* and *DDC*, respectively — ectopically using the *GAL4-UAS* system. In this approach, a gene is linked to an upstream activating sequence (*UAS*) that confers responsiveness to the transcription factor *GAL4*; expression in a panel of different *GAL4* lines leads to a variety of specific patterns of expression in various parts of the fruitfly, including the wing.

True *et al.* [4] found that, even though dopa decarboxylase has been determined to be a major component in melanin biosynthesis [7], ectopic expression of its gene was unable to produce melanization in the adult wing. None of *GAL4* lines exhibited ectopic melanization in the adult wing when crossed to a *UAS-DDC* line, even though strong ectopic expression of dopa decarboxylase was seen in the

Figure 1



Phylogenetic relationships among some selected drosophilid flies [9–11] with representative wing patterns. The letter after each species name refers to one of the wings illustrated on the right.

wing imaginal disc. In contrast, when crossed to the *UAS-TH* lines, three of the lines in which *GAL4* is expressed ubiquitously did show gradual formation of stripes in intervein regions of the adult wing. Interestingly, when *UAS-TH* and *UAS-DDC* were co-expressed under the control of *hsp70-GAL4*, the melanization patterns were more intense than with either *UAS-TH* construct alone. This suggests that, although both loci clearly play a role in melanin formation, ubiquitous expression of tyrosine hydroxylase alone is sufficient for ectopic melanization. This surprising result suggests that changes in wing melanization patterns may be under the control of a single gene.

True *et al.* [4] also tested whether the molecules necessary for melanization in adult wings are melanin precursors themselves, as opposed to hormonal signals or some

other activating character. Excised unpigmented wings from newly eclosed male members of a species with a distinctive wing melanization pattern, *D. rajasekari*, when incubated with the melanin precursors dopa and dopamine displayed the normal, male-specific melanin pattern. This experiment shows that it is the presence of melanin precursors in localized regions of the wing that is necessary for melanin patterns, not hormonal or other activation signals.

The physiological brush stroke

While the ubiquitous expression of tyrosine hydroxylase can produce ectopic melanization, the exact localization of melanization in the wing is under different control. Clues for the control of the positioning of melanization came from observations on lines expressing the *UAS-DDC/TH* constructs and also *ebony* mutant flies. In these lines, ectopic melanin is always associated with the wing veins, suggesting that melanization is dependent on veins. True *et al.* [4] used two mutants which have truncated wing veins, *veinlet* and *Vein off*, to show that intact veins are required for pigmentation in the wing. They found that *ebony veinlet* double mutants and flies in which the *UAS-TH* construct was expressed on a *Vein off* genetic background both failed to develop pigmentation patterns in areas where wing veins were deleted. True *et al.* [4] suggest that the wing veins serve as conduits for the dispersion of the precursors required for melanin production.

True *et al.* [4] also examined rare mutant lines of Hawaiian picture-winged *Drosophila* that have incomplete veins, to show that their observations extend to species other than *D. melanogaster*. They found that wing-vein mutants of *D. grimshawi* and *D. heteroneura* do not develop wild-type pigmentation in areas where wing veins have been deleted, much like the *D. melanogaster* mutant lines. Wing veins thus seem to play a phylogenetically broad role in the distribution of melanin precursors, serving to paint the wings of many species of *Drosophila*, and possibly other insects as well.

Painting evolutionary scenarios

True *et al.* [4] have shown that the process by which melanin patterns are produced in *Drosophila* appears to be under the control of a relatively simple developmental hierarchy. The action of a single gene, tyrosine hydroxylase, is sufficient to change wing melanization patterns. Reaction–diffusion models, such as those postulated by Turing and others [1–3] to explain many pigmentation patterns in vertebrates, may not be applicable to insect wings. Instead, veins in adult wings appear to directly determine the pigmentation patterns by transporting melanin precursors to the appropriate locations.

Detailed molecular and genetic studies cannot easily be done in species other than the well-characterized,

genetically tractable model organisms. But knowledge gained from work on the model systems can help understand the evolution of morphological structures in related taxa [5,8]. True *et al.* [4] have demonstrated that melanization is vein-dependent in several Drosophilidae species. Could this also be the case in other, more poorly studied drosophilid species? For example, most species in the genus *Zygothrica*, a group closely related to the subgenus *Drosophila*, have wing venation patterns that are identical to those of *D. melanogaster*, *D. rajasekari*, *D. heteroneura* and *D. grimshawi*. One newly discovered *Zygothrica* species from Ecuador, however, has three extra crossveins along the margin of the wing, each of which is pigmented (Figure 1j). Although no mutants are available for this species, the results of True *et al.* [4] suggest that melanin precursors could have been deposited by the hemolymph transport system on the basis of a previously established prepatter; prior to this study, no hypothesis for how these *Zygothrica* wing pigmentation patterns are generated could be proposed.

Other questions are raised when one begins to look beyond *D. melanogaster*. For example, how is a pigmentation prepatter as complex as that of *D. adiantola* (Figure 1a) or *D. calloptera* (Figure 1i) established at the molecular level? What regulatory interactions are required to specify where the melanin precursors are deposited? Are they deposited ubiquitously in the wing and selectively degraded in some areas before late pupal development, when the melanization pathway is initialized? If the veins transport these products into the wing, why do some veins — for example, the posterior crossvein and the apices of the long veins — seem to be pigmented more often than others? The work of True, *et al.* [4] provides an exciting first step towards answering some of these questions.

Studies such as the one by True *et al.* [4] should also be helpful for systematics. Their results suggest that wing melanization patterns have a relatively simple genetic control, and might consequently evolve relatively easily. This has implications for systematics, as it means particular characters might evolve more than once — homoplasy — and so cannot be assumed to be indicative of a phylogenetic relationship. In fact, this is what we observe in the Drosophilidae. Many species groups include species with and without pigmentation patterns, and it is clear that this character has evolved frequently throughout evolutionary time. Use of wing pigmentation patterns must, therefore, be scrutinized carefully before they are used as systematic characters. For example, even though the complex pigmentation patterns on the wings of *D. calloptera* (Figure 1i) and *D. adiantola* (Figure 1a) are somewhat similar, they would make poor characters in a higher-level analysis because, from the phylogeny in Figure 1, they are clearly not homologous. These same characters are,

however, very useful when examined at lower taxonomic levels — determining systematic relationships within the *calloptera* or picture-wing species groups.

Many evolutionary scenarios formulated on the basis of developmental data have been painted with extremely broad brushstrokes, with many assumptions made at the genetic and molecular level. As more systems, such as the wing melanization system deciphered by True *et al.* [4], become available to developmental and evolutionary biologists, there will be more evolutionary problems that can be examined in a rigorous experimental fashion and the brushstrokes used to paint evolutionary scenarios will become finer and finer.

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