Evolution in black and white: genetic control of pigment patterns in *Drosophila*

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Coloration is one of the most variable characters among animals and is a rich source of models of phenotypic evolution. The great diversity of pigment patterns in *Drosophila*, coupled with the availability of genetic approaches in both model and more exotic species, has recently spawned efforts to elucidate the genetic architecture and molecular basis of pigment pattern evolution. Pigmentation differences are often polygenic and correlate with regulatory changes in both transcription factor genes and structural genes. Understanding the developmental genetic basis of color differences in *Drosophila* could provide inroads to classic evolutionary problems such as industrial melanism, mimicry and phenotypic convergence.

> The formation of a pigment is one of the simplest of developmental processes…something essentially less complicated than the organs and tissues with which embryological research is usually concerned [1].

A key challenge in evolutionary biology is to understand the genetic and developmental basis of phenotypic diversity. A multi-disciplinary approach that combines developmental and molecular biology with ecology and population genetics holds the most promise for elucidating the mechanisms of evolutionary change. Traits that have diverged significantly among closely related species are particularly well-suited to such an integrated analysis. Insect pigmentation offers an attractive model for study because it is relatively simple at the molecular level, has ecological importance and exhibits frequent evolutionary changes. Furthermore, genetic and developmental mechanisms that control pigmentation in *Drosophila* have recently been identified. Here, we review recent work on the development, evolution and functional importance of *Drosophila* pigmentation. We explore the diversity of pigment patterns and review our current understanding of the genetic regulation of melanin patterning and synthesis in *D. melanogaster*. Next, we focus on four general questions concerning the genetic and molecular mechanisms of phenotypic evolution:

(1) Which genes underlie phenotypic divergence between species?
(2) What is the molecular nature of genetic differences that contribute to this divergence (regulatory or coding)?
(3) Are the same genes involved in intraspecific variation and interspecific differences?
(4) Are the same loci responsible for similar phenotypic changes in different evolutionary lineages?

Endless forms or variations on a theme? Pigmentation diversity in *Drosophila*

Most species of *Drosophila* and related genera have a mixture of light and dark pigments alternating in some sort of spatial pattern. The most prevalent color scheme is either black or dark brown on a tan or yellow background, but various shades of light brown and gray – sometimes with a greenish or reddish tint – are also seen in some species. Among this multitude of patterns, several recurrent elements have been observed that are shared across many taxa. Different combinations of these elements, as well as subtle variations of each element, produce the observed diversity.

The most common pattern in *Drosophilidae* is a band of dark pigment located at the posterior edge of the dorsal cuticular plate (tergite) of each abdominal segment (Fig. 1b; arrow). These bands, which give flies their characteristic striped appearance, are also modulated along the dorso-ventral axis. In many taxa, the pigment bands are either widened (Fig. 1b; arrowhead) or interrupted (Fig. 1f; arrowhead) at the dorsal midline, or near the lateral tergite margins (Fig. 1e,f; arrows). A few groups show more complex patterns, where pigment stripes are widened at several positions along the dorso-ventral axis (Fig. 1g; arrows), or broken up into several isolated spots (Fig. 1h). In many species, the pattern and/or intensity of pigmentation varies from one abdominal segment to the next (Fig. 1a,i), sometimes in a sex-specific manner (compare Fig. 1a with Fig. 1b). Finally, some species have no discernible spatial pattern, and are either uniformly dark (Fig. 1c) or uniformly light (Fig. 1d).

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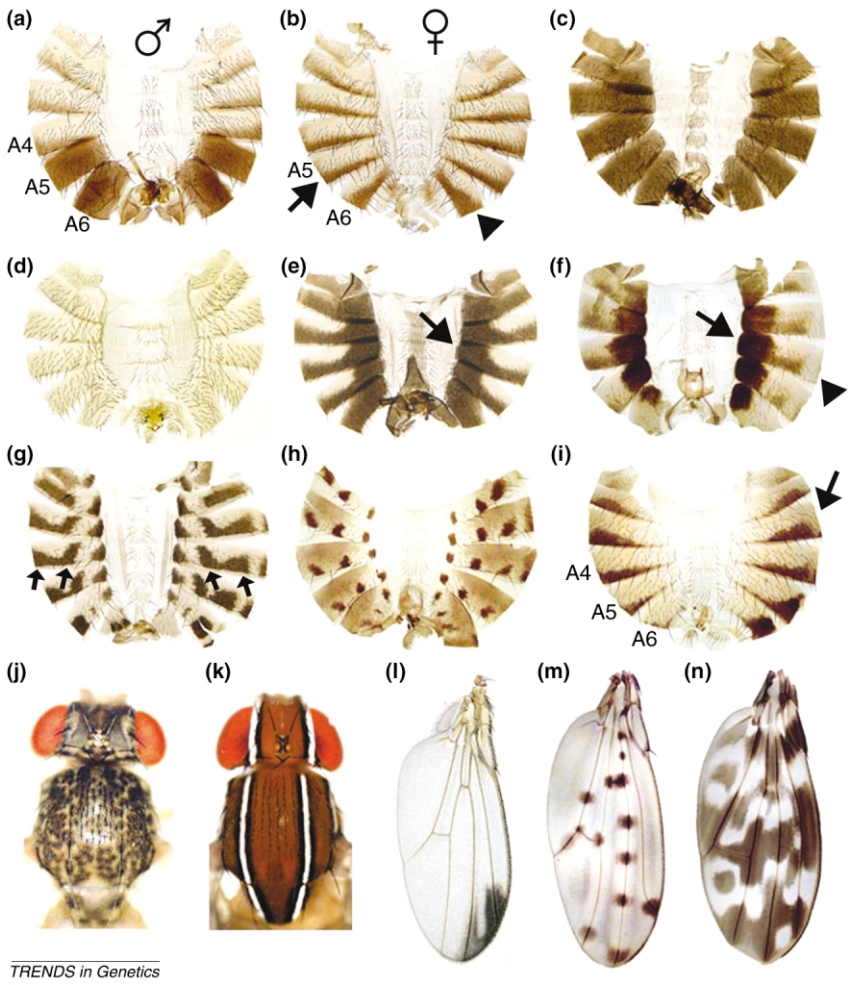

Fig. 1. Diversity of pigment patterns in *Drosophilidae*. (a–i) Abdominal pigment patterns. Adult abdominal cuticles were cut open along the dorsal midline and mounted flat as described by Duncan [65]. Anterior is up, the ventral cuticle is in the center and the dorsal cuticle is on the outside. (a) *D. melanogaster* male. (b) *D. melanogaster* female. In this classical example of sex- and segment-specific pigmentation, the last two abdominal segments (A5 and A6) are completely pigmented in males, but not in females. Note also that segmental pigment stripes (arrow) are widened near the dorsal midline (arrowhead) – a feature common to most members of the subgenus *Sophophora*. (c) A melanic species *D. mimica* (Hawaiian modified-mouthparts species group). (d) *D. ananassae* (*melanogaster* species group) completely lacks dark pigmentation. (e) In *D. saltans* (*saltans* species group), pigment bands are widened at the lateral tergite edges (arrow), as well as at the dorsal midline. (f) In *D. hydei* (*repleta* species group), pigment bands are interrupted at the dorsal midline (arrowhead), as in most members of the subgenus *Drosophila*. (g) In *D.* (*Dorsilopha*) *busckii*, pigment bands are widened and interrupted at multiple points along the dorso-ventral axis (arrows). (h) In *D. guttifera* (*quinaria* species group), pigment bands are broken up into isolated spots. (i) In *D. unipunctata* (*tripunctata* species group), pigment bands are interrupted at the dorsal midline in the anterior segments (arrow), but not in A5 or A6. (j,k) Notum and head pigmentation. (j) *D. nigrospiracula* (*repleta* species group). Each dark spot is associated with a mechanosensory bristle. (k) *Zaprionus vittiger*. The two 'racing stripes' typical of this genus coincide with the position of the dorsocentral bristles. (l–n) Wing pigmentation. (l) *D. elegans* (*melanogaster* species group). (m) In *D. guttifera* (*quinaria* species group), each wing spot is associated either with a mechanosensory organ, or with a confluence of two veins. (n) *D. crucigera* (Hawaiian picture-wing species group). Wing patterns in Hawaiian *Drosophila* do not follow any obvious morphological landmarks but are nevertheless highly reproducible from individual to individual.

Several *Drosophila* lineages have unusual pigment patterns on the notum, ranging from 'speckles' in the *repleta* group (Fig. 1j), to the dramatic 'racing stripes' of *Zaprionus* (Fig. 1k). Wing pigmentation can also take the form of relatively simple spots (Fig. 1l,m), or very complex, species-specific patterns, as seen in Hawaiian picture-wing *Drosophila* (Fig. 1n). Little is known about the development of the wing pigment patterns, except that they require diffusion of precursors from veins into the inter-vein tissue [2].

Evolution of pigment patterns involves frequent divergent and convergent changes

Evolutionary relationships among many extant *Drosophilidae* have been elucidated by recent phylogenetic studies based on DNA sequences [3]. These phylogenies enable reconstruction of the history of changes in pigmentation in different evolutionary lineages. In some clades, such as the *willistoni* and *obscura* species groups, pigmentation is generally static; all their members are pigmented almost identically. However, in other lineages, dramatic differences in pigmentation have evolved among recently diverged species. For example, wing pigmentation has appeared or disappeared at least six times during the evolution of 18 members of the *melanogaster* species group [4]. Sex- and segment-specific abdominal pigment patterns within the *montium* subgroup have also been gained and lost many times (A. Kopp, unpublished). One interesting example of pigmentation divergence exists among the Caribbean representatives of the *cardini* species group. In this clade, pigmentation shows no correlation with species phylogeny, but instead follows their geographic distribution [5].

For all of their diversity, pigment patterns show many recurrent themes among *Drosophilidae*. For example, some traits, including melanism, sexually dimorphic abdominal pigmentation, and wing pigmentation, have clearly evolved independently in multiple, distantly related lineages (Fig. 2). An outstanding question in evolutionary genetics is whether the same genes are responsible for the origin of similar phenotypes in different evolutionary lineages. Convergent pigment patterns among *Drosophila* species provide an excellent opportunity to address this and other long-standing evolutionary questions. In the following sections, we discuss how our growing knowledge of the genetics and development of *D. melanogaster* has been used to investigate the genetic and molecular mechanisms of pigmentation evolution among *Drosophila* species.

Genetic control of pigmentation development in *D. melanogaster*

Like most traits, pigmentation is controlled by regulatory genes, such as transcription factors, which control the expression of other genes, and structural genes, many of which encode the enzymes that comprise the biochemical pathways used for pigment synthesis. The same sets of pigments and structural genes are used to form each part of the overall pattern, but different regulatory genes might control their expression in different body regions.

Structural genes that function in pigment synthesis

Cuticular pigments of *Drosophila* are polymerized derivates of dopa (dihydroxyphenylalanine) and dopamine (dihydroxyphenylethylamine), which are synthesized in turn from the amino acid tyrosine (reviewed in [6]). Pigment precursors are produced and secreted by epidermal cells and incorporated into the developing cuticle during late pupal stages [7]. Our current understanding of the biochemical pathway that produces cuticular pigments is represented in Fig. 3. The activity of most enzymes in this pathway is limited to the cells in which they are expressed, and secreted pigment precursors only move a few cell diameters [2,8]. The color of each cuticular area is determined by the balance of enzymatic activities in that area [9]. The diversification of pigment patterns in *Drosophila* presumably reflects evolutionary changes in the deployment or activity of these enzymes.

Regulatory genes that control pigment patterning

Spatial patterning of pigmentation has been studied most thoroughly in the abdomen of *D. melanogaster*. In most abdominal segments, the dorsal cuticular plate (tergite) bears a posterior stripe of dark pigment (Fig. 4a). This pattern is regulated – probably indirectly – by the T-Box transcription factor *optomotor-blind* (*omb*), which is, in turn, controlled by the Hedgehog (Hh) signaling pathway [10–12]. Pigment stripes are wider at the dorsal midline and taper off towards the lateral edges of the tergite (Fig. 4a). This pattern is controlled by the Decapentaplegic (Dpp) signaling pathway, which, together with Wingless and epidermal growth factor receptor signaling, is responsible for the dorso-ventral patterning of abdominal segments [13]. The wide variety of spots and stripes seen in other species (Fig. 1) might reflect different responses to the same spatial cues.

Drosophila melanogaster also displays an additional sex- and segment-specific pattern that does not depend on *omb*. The two most posterior abdominal segments in males have uniform black pigmentation that masks the usual pigment stripes (Fig. 1a). This male-specific pigmentation is repressed in females by the expression of two related transcription factors encoded by the *bric a brac* (*bab*) locus [14,15]. In males, *bab* expression is repressed in these segments by the HOX protein Abdominal-B, but, in females, expression of the female-specific isoform of the sex determination protein Doublesex overcomes this repression [15] (Fig. 4b). Changing the expression of regulatory genes, such as *omb* and *bab*, can alter pigment patterns [15,16], presumably by altering the expression patterns of structural genes. Such changes could have played an important part in the diversification of pigment patterns.

Regulation of gene expression by modular enhancers

Many of the pattern elements illustrated in Fig. 1 are inherited individually in interspecific crosses [17–19], indicating that these patterns are regulated independently during development. In addition, several mutants have been identified that alter one part of the pigment pattern without affecting any other [15,16,20]. This independence of different pattern elements appears to reflect the modular organization of the genetic regulatory machinery that controls pigmentation development.

Structural genes involved in pigment synthesis are expressed in many areas of the developing epidermis, yet, in the course of evolution, different aspects of the pigment pattern have clearly evolved independently of each other. How is independent regulation of the same structural genes achieved between different parts of the developing animal? The answer lies in the modular control of gene expression [21]. For instance, the *yellow* locus contains five independent *cis*-regulatory elements (enhancers) that control its expression in the developing body, wings, bristles, larval mouthparts and denticle belts [22,23]. There is also evidence for a similar modular organization of the *cis*-regulatory regions of other enzyme genes, including *pale*, *Ddc* and *ebony* [24–27]. Because each *cis*-regulatory element is independent, sequence changes in one enhancer do not affect the regulatory function of other enhancers, enabling gene expression to evolve independently in each body part.

An extra layer of flexibility is added by the complex control of the regulatory genes that direct pigment development. Most regulatory proteins are themselves controlled by multiple, independently regulated enhancers. In particular, *omb*, *bab* and *dpp* contain distinct enhancers that drive their expression in the pupal abdomen, whereas *Abd-B* expression is controlled by segment-specific regulatory elements [13,28,29] (A. Kopp and S.B. Carroll, unpublished). This modular control of genes at multiple levels in the regulatory hierarchy that

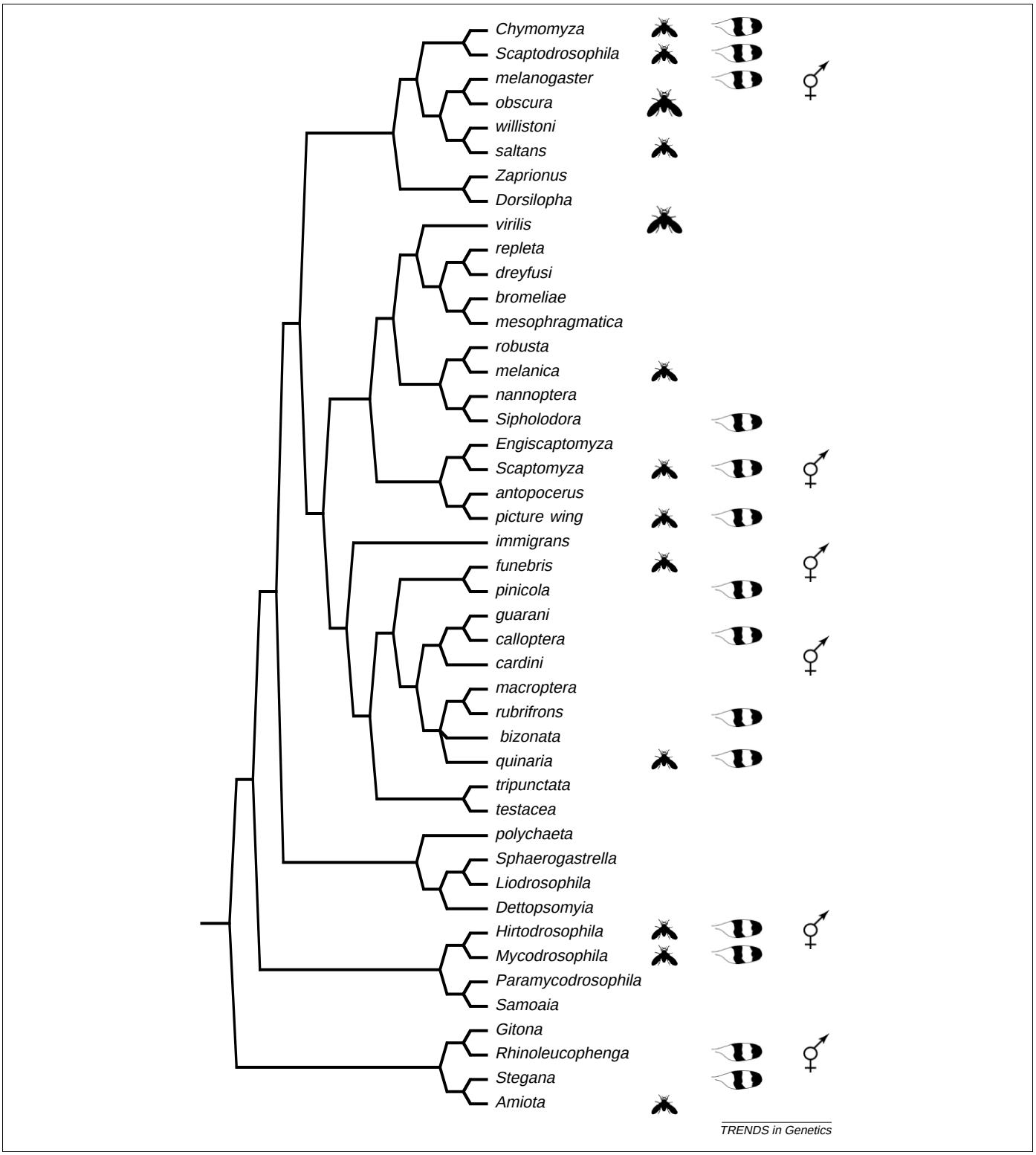

Fig. 2. Convergent evolution of pigment patterns in *Drosophilidae*. Reconstruction is based on the molecular phylogeny of Remsen and O'Grady [3]. Taxon names beginning with a capital letter denote genera and subgenera; names of species groups are in lower case. Black fly symbols indicate that the lineage contains melanic species; a larger symbol indicates that all or most of the lineage is melanic, whereas a smaller fly indicates that the lineage is mostly lightly pigmented, but contains some melanics. A wing symbol indicates that some species show patterned wing pigmentation. This excludes species with clouded crossveins, which are present in many other lineages. Finally, the male and female symbols indicate the presence of species with especially pronounced sexually dimorphic pigmentation (more subtle sex differences are seen in many other species). Note that these summaries are probably not exhaustive because the authors are not familiar with all of the >3000 described species of *Drosophilidae*.

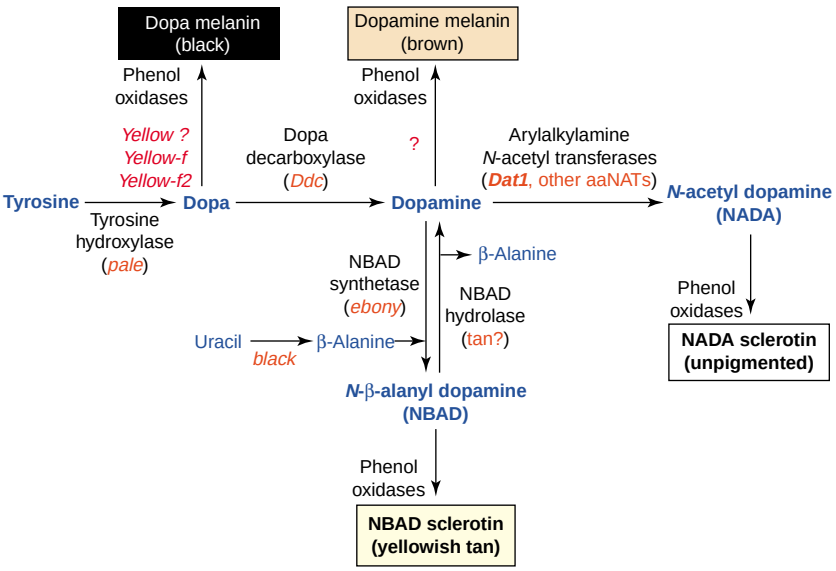

Fig. 3. Pigment metabolism in *Drosophila melanogaster* (based on the work of Wright [6] and Wittkopp *et al.* [9]). Pigment precursors are shown in blue, enzymes are shown in black, and the genes that encode them are shown in red. The synthesis of all pigments begins with the conversion of tyrosine to dopa (dihydroxyphenylalanine) by the enzyme tyrosine hydroxylase, encoded by the *pale* gene. Some dopa is then converted to black dopa melanin by the activities of the Yellow, Yellow-f1 and Yellow-f2 proteins [66]. In another branch of the pathway, dopa decarboxylase (Ddc) converts dopa to dopamine, which serves as a precursor for brown melanin [9,67]. Alternatively, dopamine can be shunted away from melanin synthesis and towards the production of light pigments. The product of the *ebony* gene converts dopamine to N-β-alanyl dopamine (NBAD), which is used in the production of yellow sclerotin. This reaction is reversible, and some NBAD is converted back into dopamine by an NBAD hydrolase, which is thought to be encoded by the *tan* locus [6]. Finally, a family of arylalkylamine-N-acetyl transferases (aaNATs) converts dopamine to N-acetyl dopamine, which serves as a precursor for colorless or transparent sclerotin [6,68]. Some genes involved in pigment synthesis remain to be identified (indicated by question marks).

orchestrates pigment development might facilitate the divergence of pigmentation among closely related taxa.

Molecular mechanisms of pigmentation divergence

In *Drosophila*, the overall body plan is well-conserved, and many developmental mechanisms appear to be similar among distantly related species (e.g. [30–33]). The biochemistry of pigment synthesis, and the enzymes involved in this process are also highly conserved, not only among *Drosophilidae*, but also in other insects [34]. Thus, the diversity of pigment patterns among related species might reflect differences in the deployment of similar sets of regulatory and structural genes. *Drosophila* pigmentation is one of very few phenotypic traits in any animal for which both regulatory and structural genes have been identified, enabling us to ask for the first time whether phenotypic diversification has been driven by changes in the regulatory genes at the top of the developmental hierarchy, or in their structural gene targets, or both.

Differences in expression of both regulatory and structural genes correlate with divergent pigmentation

Most members of the *melanogaster* species group have sexually dimorphic pigmentation, where the last two abdominal segments are fully melanized in males, but not in females (Fig. 1a,b). This pattern is a fairly recent evolutionary innovation; in most other *Drosophila* species, males and females share similar pigmentation (but see Fig. 2 for exceptions). In *D. melanogaster*, development of the sexually dimorphic pigment pattern is controlled by the *bab* locus [15]. Comparison of *bab* expression among 26 species shows that, in most species with male-specific pigmentation, *bab* is expressed in a sex- and segment-specific pattern similar to that seen *D. melanogaster* [15]. In sexually monomorphic species, however, *bab* is expressed equally in both sexes and in all abdominal segments. This suggests that evolutionary changes in the regulation of *bab* might have been important for the origin of sexually dimorphic pigmentation. Recently, it has been shown that modulation of *bab* expression is correlated with diverse pigmentation patterns in several *Drosophila* clades [35]. It appears that changes in *bab* regulation have evolved independently to shape convergent and divergent melanin patterns.

Differences in the overall degree of abdominal pigmentation have been shown to correlate with evolutionary changes in the expression of structural genes, most notably, *yellow* and *ebony*. Yellow expression in the developing abdominal epidermis in five *Drosophila* species was strongly correlated with the distribution and intensity of black melanin in all but one of them [18,36]. This fifth species (*D. novamexicana*) expressed moderate levels of Yellow protein despite the production of very little black pigment. However, expression of the Ebony protein in *D. novamexicana* was elevated relative to its darker sister species *D. americana* [18]. Yellow and Ebony have opposite effects on cuticle color, and increased Ebony expression can counteract the ability of Yellow to promote black pigment formation [9]. The observed increase in Ebony expression might therefore shift the balance between black and yellow pigments to produce the light phenotype of *D. novamexicana*. Changes in the expression of *yellow*, *ebony* and possibly other structural genes might also have

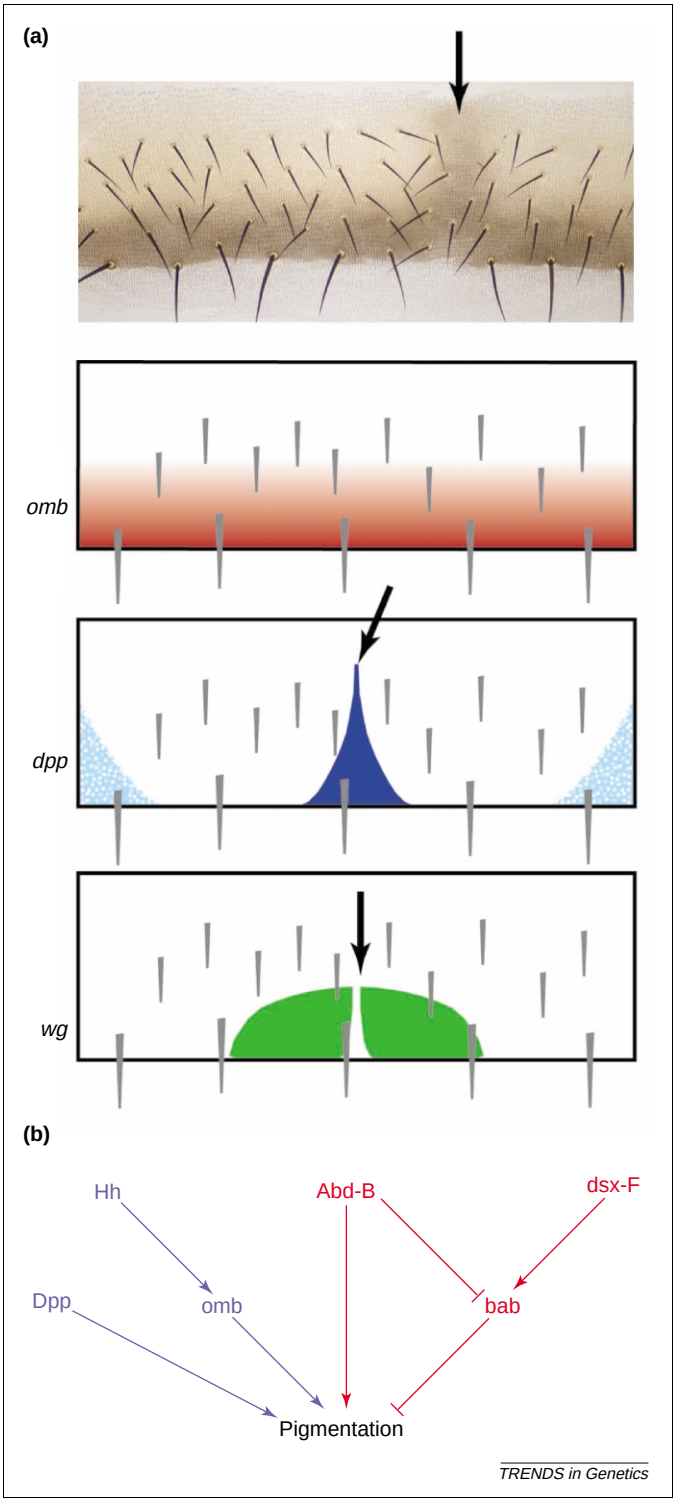

Fig. 4. Spatial regulation of abdominal pigment patterns in *Drosophilia melanogaster*. (a) Expression of *omb* (red), *dpp* (blue) and *wg* (green) in abdominal tergites (dorsal cuticular plates that correspond to the anterior compartment of each segment). Pigmentation of a typical tergite is shown in the top panel for comparison. Note the posterior pigment band, widened at the dorsal midline (arrow). The dorsal midline is also marked by arrows in the *dpp* and *wg* panels. *omb* is expressed in a gradient at the posterior edge of the tergite. *dpp* is expressed along the dorsal midline and, transiently, at the lateral tergite margin (light blue). *wg* is expressed in broad medial domains flanking the *dpp* stripe. Dpp signaling is required for the mid-dorsal widening of the pigment band, and Wg, together with Epidermal Growth Factor signaling, promotes more lateral tergite fate [13]. (b) Genetic control of abdominal pigment patterns. The sexually dimorphic pathway is shown in red, and the sexually monomorphic pathways are shown in blue (see main text for details). Arrows indicate positive regulatory interactions, blunt arrows indicate negative regulatory interactions.

been involved in the evolution of wing pigmentation. In several species with male-specific wing pigmentation (Fig. 1), cells that produce the pigment spot have increased Yellow expression and decreased Ebony expression [4,9].

In all these studies, the divergence of pigment patterns was correlated with differences in the expression of either regulatory or structural genes. Although evolutionary changes in protein function (e.g. enzymatic activity or DNA-binding specificity) have not been ruled out, it appears that *Drosophila* pigmentation evolves largely through changes in gene regulation.

Both cis- *and* trans-*regulatory changes underlie divergent gene expression patterns*

Because regulatory proteins control, either directly or indirectly, the expression of enzymes involved in pigment synthesis, observed interspecific differences in the expression of structural genes might be merely a consequence of changing expression patterns of their upstream regulators. Alternatively, divergent activities of the *cis*-regulatory regions of the structural genes themselves might be responsible. These two mechanisms can be distinguished by comparing the expression of orthologous genes in transgenic animals of different species (Box 1). This approach has been used to investigate the genetic basis of differences in Yellow expression between *Drosophila* species [36]. A comparison of *yellow cis*-regulatory regions from *D. melanogaster* with *D. subobscura* showed that sequence differences within a tissue-specific enhancer were responsible for species-specific expression patterns. However, reciprocal transformations of the *yellow* genes between more distant relatives (*D. melanogaster* and *D. virilis*) indicate that the genetic changes responsible for differences in expression are located both within the *yellow* gene itself (presumably in *cis*-regulatory regions) and elsewhere in the genome. That is, both *cis*- and *trans*-regulatory changes contribute to the interspecific divergence of Yellow expression.

Genetic basis of species divergence

Comparing gene expression between species can reveal how developmental processes have diverged, but identifying specific genetic changes responsible for interspecific differences remains a challenging task. Linkage-based mapping offers a more direct way to identify the genetic basis of phenotypic differences. Genetic analysis has been used to estimate the number of loci responsible for differences in pigmentation in several pairs of interfertile *Drosophila* species. In many cases, the differences were found to be polygenic, whereas in other species, a single Mendelian factor appears to be responsible (Table 1). Two of these studies used molecular markers to estimate gene number more accurately and to identify the genomic regions that contain quantitative trait loci (QTLs) that contribute to interspecific divergence. Llopart *et al.* found evidence for at least five such loci in crosses between two members of the *melanogaster* species subgroup, *D. yakuba* and *D. santomea* [37]. Similarly, Wittkopp *et al.* detected a minimum of four loci underlying differences in abdominal pigmentation between *D. americana* and *D. novamexicana* in the *virilis* species group [18]. Both studies also tested

Box 1. Identifying the molecular basis of divergent gene expression patterns

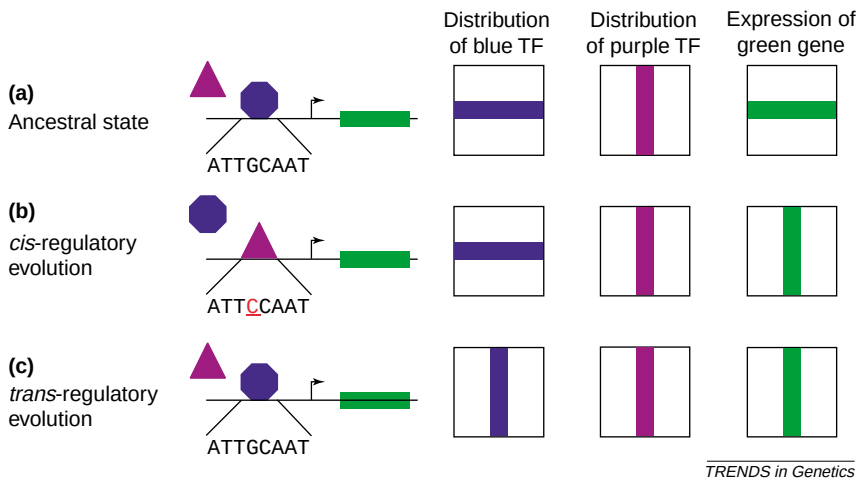

Fig. I. (a) Simplified schematic of gene regulation. Transcription factor (TF) proteins, such as the one shown in blue, regulate gene expression by binding to *cis*-regulatory DNA sequences. Within a cellular field, the distribution of a TF (blue) controls the distribution of its target gene (green). (b) Sequence changes in the *cis*-regulatory regions can cause the gene to be regulated by different TFs. If the distribution of these new factors is different (purple), a change in gene expression can result (green). (c) Alternatively, the distribution of a *trans*-regulatory factor (blue) can change, while its target *cis*-regulatory sequences remain conserved. This would also result in a novel gene expression pattern (green). Shapes represent hypothetical transcription factors, arrows indicate transcription start sites, and green blocks represent the coding region of the gene. Expression patterns are shown in a schematic cellular field. For simplicity, a single transcription factor is shown, although most genes are actually under the combinatorial control of multiple transcription factors.

Evolutionary changes in gene expression have played a prominent role in phenotypic divergence among taxa [21,69]. However, specific molecular changes responsible for divergent gene expression patterns have only been identified in a few cases (reviewed in [70]). Gene expression is controlled by the interaction of *trans*-acting transcription factors with *cis*-regulatory DNA sequences. Thus, changes in gene expression can arise from differences in either the presence or activity of *trans*-regulatory proteins or in the *cis*-regulatory sequences to which they bind (Fig. I). Comparing the expression of divergently expressed, orthologous genes in a common genetic background can distinguish between *cis*- and *trans*-regulatory changes.

One powerful method for making such comparisons involves the analysis of transgenic animals that carry heterologous transgenes [70]. Assume Gene X is expressed differently in Species A and Species B (Fig. IIa). Orthologous genes from the two species (X_A and X_B), including all of their *cis*-regulatory regions, are transformed separately into one of the species (e.g. Species A). As the two transformants of Species A differ only in the transgene that they carry, the expression of both transgenes (X_A and X_B) is controlled by the same *trans*-regulatory factors. If the expression of X_A and X_B in Species A differs, we can infer the presence of evolutionary changes within the *cis*-regulatory sequences included in the transgene (Fig. IIb). Furthermore, if the expression of X_B in Species A is identical to its native expression in Species B, then all of the *trans*-regulatory factors required for the expression of X_B are conserved between the two species. Reciprocal transformation of both genes into Species B is necessary to test if the *trans*-regulatory factors controlling the expression of X_A are conserved. Such reciprocal transformations, made possible by recent technological advances [71], can identify genetic differences that are not detected by the comparison of orthologous genes in a single species [36].

Cis- and *trans*-regulatory changes are not mutually exclusive and, in most studies performed to date, both appear to contribute to divergent expression patterns among *Drosophila* [70]. Furthermore, the description of genetic changes as *cis* or *trans* is relative to the gene whose expression is being compared. In reality, *trans*-regulatory effects might actually be caused by *cis*-regulatory changes in genes that encode transcription factors or other regulatory proteins that alter the expression of the gene tested in the transgenic experiments. Working progressively from divergent expression patterns to the specific molecular sequence changes responsible for them will eventually lead to identification of the genetic changes that contribute to phenotypic differences.

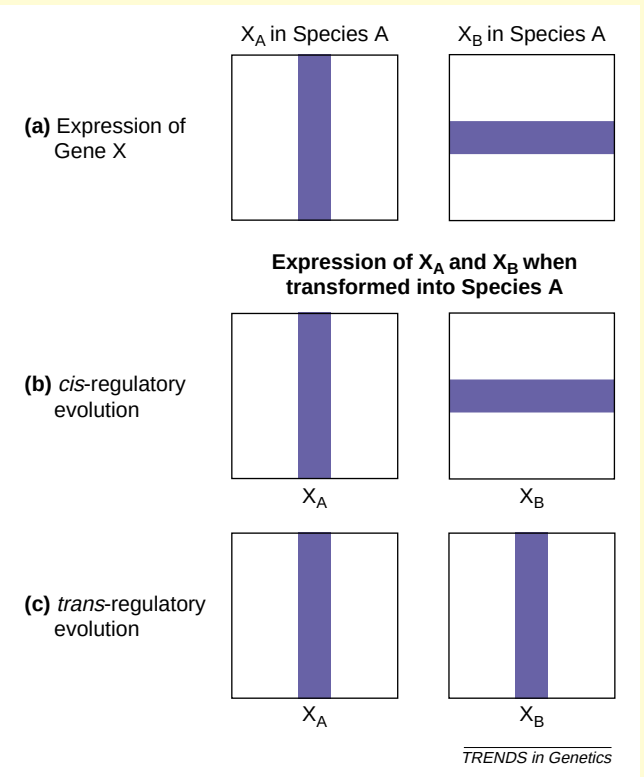

Fig. II. (a) Divergent expression patterns of Gene X in Species A and Species B. (b) Species-specific expression patterns of X_A and X_B are maintained when transformed into Species A if *cis*-regulatory changes are solely responsible for the divergent patterns. (c) By contrast, if the *cis*-regulatory sequences are conserved between X_A and X_B, but the distribution of *trans*-regulatory factors that bind to them has changed between the two species, then the expression of the heterologous gene (X_B) should resemble the expression of the endogenous gene (X_A). If divergent expression patterns are caused by both *cis*- and *trans*-regulatory evolution, expression of a heterologous transgene will not recapitulate the native expression pattern from either species. Reciprocal transformations of orthologous genes can begin to distinguish between the relative contributions of *cis*- and *trans*- changes.

Table 1. Genetic basis of interspecific differences in pigmentation

Species pair	Trait(s)	Estimated no. of genes	Refs
americana/novamexicana	Abdominal dorsal midline	Four or more	[17,18]
	Abdominal tergites	Four or more	
santomea/yakuba	Total body pigmentation	Five or more	[37]
silvestris/heteroneura	Multiple traits (abdomen, thorax, legs, wings)	Several	[52]
nigrodunni/arawakana	Multiple traits (abdomen)	Several	[19]
macrospina/subfunebris	Total body pigmentation	One major locus	[53]
bipectinata/malerkotliana	Abdominal segments 4–6	Two or more	[a]
jambulina/watanabei	Abdominal segment 6	One locus	[a]

[a]A. Kopp, unpublished.

the involvement of specific candidate genes that were known to function in pigment patterning or synthesis. In almost all cases, the candidate loci showed no association with interspecific differences. The sole exception was the *ebony* gene, which was associated with differences in pigmentation between *D. americana* and *D. novamexicana* [18]. Differences in Ebony expression that correlate with pigmentation also exist between these species, further supporting the genetic association [18].

Genetic analysis of interspecific pigmentation differences is still in its early stages, and many important questions remain to be answered. The small number of markers used in the studies to date only places a lower limit on the number of genes responsible for differences in pigmentation. So far, it has not been possible to identify these genes at the molecular level, or even to determine their number precisely. Moreover, the selective genotyping strategy used makes it impossible to assess either the relative contribution of each locus, or the effects of interactions among loci [18,37]. This is an important question because conditional modifiers of pigmentation have been found in both wild populations [38] and laboratory studies [36], suggesting that epistatic gene interactions play a significant role in the evolution of pigment patterns.

Intraspecific variation in pigmentation

Frequent differences in pigmentation among closely related species suggest the existence of abundant sources of genetic variation within species. Indeed, variation in the pattern or intensity of pigmentation has been reported for many species from a variety of evolutionary lineages (Table 2). As with interspecific differences, both polygenic and monogenic inheritance has been observed within species. The most tantalizing question is whether this intraspecific variation serves as the raw material for species divergence. If this is the case, then the intra- and inter-specific differences should share a similar genetic basis.

This prediction appears to be correct in at least some taxa. In the *montium* subgroup, an identical, discrete polymorphism in female pigmentation is seen in several species, some of them distantly related. In all cases, this polymorphism is controlled by a single autosomal locus [39], which maps to roughly the same genomic region in the two species that were tested (A. Kopp, unpublished). But perhaps the most convincing example comes from *D. melanogaster*. This species has sexually dimorphic pigmentation that is controlled by the *bab* locus, and that evolved at the base of the *melanogaster* species group [15]. Recently, it was found that ~60% of the genetic variation in the extent of sexual dimorphism in a single natural population of *D. melanogaster* map to the *bab* locus [40]. Identification of the genes responsible for phenotypic differences within and among species will be necessary to connect the genetic variation segregating in populations with genetic changes underlying species divergence. In the future, we might be able to reconstruct the history of molecular changes that lead to phenotypic diversification, and better understand the roles of selection, population dynamics, and biogeographic events in shaping phenotypic evolution.

Why does pigmentation diverge?

What forces drive the divergence of pigment patterns? Do they evolve neutrally, or do the multitude of colors, stripes, and spots serve an essential biological purpose? Why does pigmentation differ among species? Answers to these questions will require a better understanding of

Table 2. Genetic basis of intraspecific differences in pigmentation

Species	Trait(s)	Estimated no. of genes	Refs
Melanogaster	Abdominal segment 6	Two large- and several weaker-effect QTLs[b]	[40,54]
elegans	Total body pigmentation	One major locus	[51,55,56]
Auraria	Abdominal segment 6	One locus	[39,57]
jambulina	Abdominal segment 6	One locus	[39,58]
kikkawai	Abdominal segment 6	One locus	[39,59]
Rufa	Abdominal segment 6	One locus	[39]
pectinifera	Abdominal segment 6	One locus	[a]
Serrata	Abdominal segment 6	One locus	[a]
malerkotliana	Abdominal segments 4–6	Two or more	[a]
polymorpha	Abdominal tergites	One major locus plus modifiers	[60–63]
Scaptomyza pallida	Notum and abdomen	One major locus	[64]

[a]A. Kopp, unpublished.
[b]Abbreviation: QTLs, quantitative trait loci.

Drosophila ecology than we have today, but several potential factors have been suggested.

Based on field observations and laboratory studies, pigmentation has been proposed to play a role in thermoregulation [41–43] and camouflage [44,45], as well as in resistance to desiccation (J. Brisson, unpublished), ultraviolet radiation [5] and (indirectly) to parasite infection (J. Jaenike, unpublished). However, none of the proposed ecological factors show an absolute correlation with pigment phenotype. For example, *D. melanogaster* and *D. kikkawai* tend to be darker in colder climates, yet many melanic species are found in the tropics. Similarly, whereas the yellow coloration of some flower-feeding species (such as *D. flavohirta*) helps them to blend in with their food source [45,46], other species that feed on lightly colored flowers (e.g. *D. elegans* and *D. gunungcola*) have dark pigmentation that makes them easily noticeable [47,48].

Some of the most elaborate pigment patterns do not appear to correlate with any obvious ecological factors. These traits could be involved in mate choice, courtship behavior and sexual selection. Sex-specific pigment patterns, especially the male wing spots displayed prominently during courtship, are particularly good candidates for traits under sexual selection. To date, however, attempts to test whether differences in pigmentation play a role in sexual selection have mainly produced negative results [37,49–51], although the role of wing pigmentation has not been tested directly.

It is clear from these studies is that no single selective force can provide a universal explanation for the evolution of pigmentation. Rather, different ecological factors have probably dominated in different species, in different populations, and at different times during evolution. Elucidating the functional importance of various pigment patterns is a crucial step towards understanding the selective pressures that shaped their evolution.

General insights into the molecular genetics of phenotypic evolution

The main motivation behind the pursuit of the molecular genetic mechanisms underlying the diversification of pigment patterns is to gain general insights into evolutionary processes. The development of pigmentation shares many similarities with genetic regulatory hierarchies that govern the formation of more complex traits, and we anticipate that lessons from the studies of pigmentation will shape our understanding of the types of molecular changes involved in phenotypic evolution.

The identification of specific genes involved in the development and evolution of pigmentation opens up two exciting avenues for evolutionary biology. First, these genes are candidate loci for traits in non-model species that present especially interesting examples of such evolutionary phenomena as convergence, industrial melanism, and Batesian and Mullerian mimicry. Second, because pigmentation varies within species and can respond rapidly to selection (J. Jaenike, unpublished), these genes provide an opportunity to study changes in development in a population-genetic framework. Analysis of allele genealogies and frequencies in populations from different geographic regions that experience different kinds and strengths of selection, together with an understanding of the molecular basis of the functional differences between alleles, offers the prospect of a fully integrated picture of evolution – from the nucleotide level, to the individual, to whole populations.

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