Genetic control and evolution of sexually dimorphic characters in *Drosophila*

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Sexually dimorphic abdominal pigmentation and segment morphology evolved recently in the *melanogaster* species group of the fruitfly *Drosophila*. Here we show that these traits are controlled by the *bric-a-brac* (*bab*) gene, which integrates regulatory inputs from the homeotic and sex-determination pathways. *bab* expression is modulated segment- and sex-specifically in sexually dimorphic species, but is uniform in sexually monomorphic species. We suggest that *bab* has an ancestral homeotic function, and that regulatory changes at the *bab* locus played a key role in the evolution of sexual dimorphism. Pigmentation patterns specified by *bab* affect mating preferences, suggesting that sexual selection has contributed to the evolution of *bab* regulation.

A key challenge in evolutionary biology is to identify genetic events responsible for morphological change, and to understand how changes at the molecular level affect development and translate into phenotypic diversity. To achieve this, two distinct approaches have been pursued in recent years. First, comparative studies have revealed strong correlations between the expression patterns of individual regulatory genes during development and differences in morphology[1–7]. Second, direct genetic analysis has been used to estimate the number and identity of genetic loci that contribute to morphological variation within and between species[8–10]. Despite their respective successes, the two approaches remain far apart because of their different scales of analysis. Comparative studies have concentrated mainly on slowly evolving traits among high-level taxa, but genetic analyses are only possible among closely related species that produce viable and fertile hybrids.

Our approach to bridging this gap between evolutionary genetics and comparative embryology is to analyse and compare the development of rapidly evolving morphological traits. In many animals, secondary sexual characteristics evolve rapidly[11,12], making them good candidates for analysis. One such character in *Drosophila* is the pigmentation of adult abdominal segments. In *D. melanogaster*, abdominal pigmentation is sexually dimorphic. Segments 1 to 6 in females and 1 to 4 in males carry only a posterior stripe of dark pigment. However, segments 5 and 6 (A5 and A6) in males are completely pigmented (Fig. 1a and b), giving the species its name. This pattern is of recent evolutionary origin; in most *Drosophila* species, male-specific pigmentation is absent, so that females and males are pigmented identically (Fig. 1c and d). To understand how this new pattern originated and evolved, we have characterized the regulatory circuit that controls its development, and compared its operation in sexually dimorphic and monomorphic species.

Genetic control of male-specific pigmentation

The non-sex-specific striped pigmentation, which is present in most *Drosophila* species, is controlled by the transcription factor *optomotor-blind* (*omb*)[13]. In *omb* mutants, all pigment is lost from segments A1–A4 in males (Fig. 2a), and from all abdominal segments in females (not shown). However, we find that omb^{-} males have fully pigmented A6, and mostly pigmented A5 (Fig. 2a). Thus, the ancestral striped pigmentation and the newly evolved male-specific pigmentation are controlled by separate genetic pathways.

The dark pigmentation of A5 and A6 in males is controlled by the homeotic gene *Abdominal-B* (*Abd-B*)[14,15]. In the pupal abdomen, *Abd-B* is expressed at progressively higher levels in A5, A6 and A7, but is absent from the more anterior segments (Fig. 3a). Ectopic expression of *Abd-B* in A3 and A4 expands male-specific pigmentation to these segments[14–17] (Fig. 2b). Conversely, loss of *Abd-B* expression from A5–A7 (ref. 18) eliminates male-specific pigmentation, but has no effect on the non-sex-specific pigment stripes (Fig. 2c). *Abd-B* is the primary activator of male pigmentation, but another homeotic gene, *abdominal-A* (*abd-A*), is expressed in A2–A7 (Fig. 3b) and contributes to the specification of A5 and A6 identities[14–16].

The development of sexually dimorphic external characteristics is controlled by the *doublesex* (*dsx*) gene[19,20]. Alternative splicing of the *dsx* transcript produces a male-specific product in males (*dsxM*), and a female-specific product in females (*dsxF*)[21,22]. Loss of *dsx* function in females results in the development of male-like pigmentation (Fig. 2d), which can be suppressed by heat-shock *dsxF* transgenes[23,24]. Male-specific pigmentation is therefore expressed by default, and must be actively repressed by *dsxF*.

Thus, the development of sexually dimorphic pigmentation requires integration of homeotic and sex determination gene inputs. In investigating how this integration is achieved, we discovered a newly evolved genetic circuit that appears to be responsible for the origin of male-specific pigmentation.

bric-a-brac represses male-specific pigmentation

A gene near the left tip of the third chromosome contributes to the

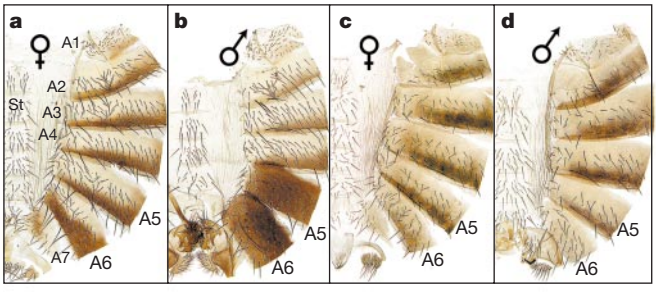

Figure 1 Sexually dimorphic pigmentation is of recent evolutionary origin. *D. melanogaster* female (**a**) and male (**b**); and *D. willistoni* female (**c**) and male (**d**). Most abdominal segments contain pigmented dorsal and unpigmented ventral cuticular plates (tergite (Ter) and sternite (St), respectively) (see Fig. 6a and b for more detail). In the ancestral condition, pigmentation is similar in males and females (**c**, **d**). In the derived condition, abdominal segments 5 and 6 (A5 and A6) are fully pigmented in the male (**b**), but not in the female (**a**).

variation in female abdominal pigmentation[25]. In investigating this genetic region, we found that loss of one copy of the *bric-a-brac* (*bab*) locus results in the development of male-specific pigmentation in females (Fig. 2e), but has no effect on the male abdomen. Ectopic pigmentation in heterozygous *bab* females is suppressed by reducing the dosage of *Abd-B* (Fig. 2f), but is not eliminated by loss of *omb* (not shown). This suggests that bab^{+} represses the development of male-specific pigmentation in females by opposing the function of *Abd-B*. The *bab* locus contains two closely related genes, *bab1* and *bab2*, which encode putative transcription factors with multiple roles in development[26,27]. Ectopic pigmentation in females increases in the order *bab1*/+ < *bab1*/*bab1* < *bab1bab2*/+ < *bab1bab2*/*bab1* (not shown), indicating that both genes are involved

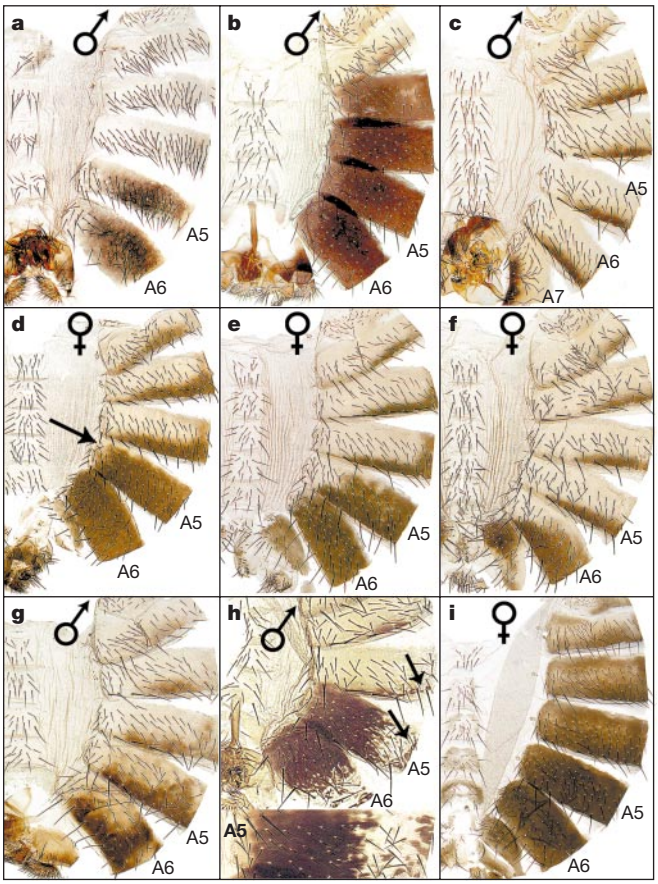

Figure 2 Control of male-specific pigmentation by *Abd-B*, *dsx* and *bab*. **a**, Male hemizygous for the *omb* null mutant $l(1)omb^{282}$ lacks segmental pigment stripes but retains pigmentation in A5 and A6. **b**, In $Abd\text{-}B^{Mcp}$ $Abd\text{-}B^{Sab}$ homozygous males, male-specific pigmentation is expanded into A3 and A4. **c**, In Df(3R)RS4–8/Df(3R)RS1–98 males, loss of *Abd-B* function from A5–A7 eliminates male-specific pigmentation, but has no effect on the non-sex-specific striped pigmentation (see also Fig. 6c). **d**, dsx^{1}/Df(3R)dsx2D chromosomal females show male-like pigmentation of A5 and A6, with the exception of a small anterior-lateral margin of A5 (arrow). **e**, bab^{Ar07}/+ female shows male-specific pigmentation of A5 and A6. **f**, *bab* phenotype is suppressed by reduction of *Abd-B* dosage in bab^{Ar07}/ Df(3R)RS4–8 females. **g**, Low-level *bab* expression in UAS-$bab2^{4-66}$ in the absence of a GAL4 driver results in the loss of male-specific pigmentation, but has no effect on the non-sex-specific pigmentation. **h**, High-level *bab* expression in *pnr*-GAL4/ UAS-$bab2^{4-51}$ males results in the loss of both male-specific and striped pigmentation (arrows); the latter effect is seen in females as well. An identical phenotype is caused by misexpression of *bab1*. The loss of male-specific pigmentation in this and other GAL4/UAS-*bab* combinations is enhanced by *abd-A Abd-B* deficiencies, and partly suppressed by *abd-A Abd-B* duplications (not shown). **i**, bab^{Ar07}/bab^{Ar07} female. Ectopic pigmentation is seen in A2–A7 tergites, A4–A7 sternites, and occasionally in the A1 tergite. An identical pigmentation phenotype is seen in males (not shown).

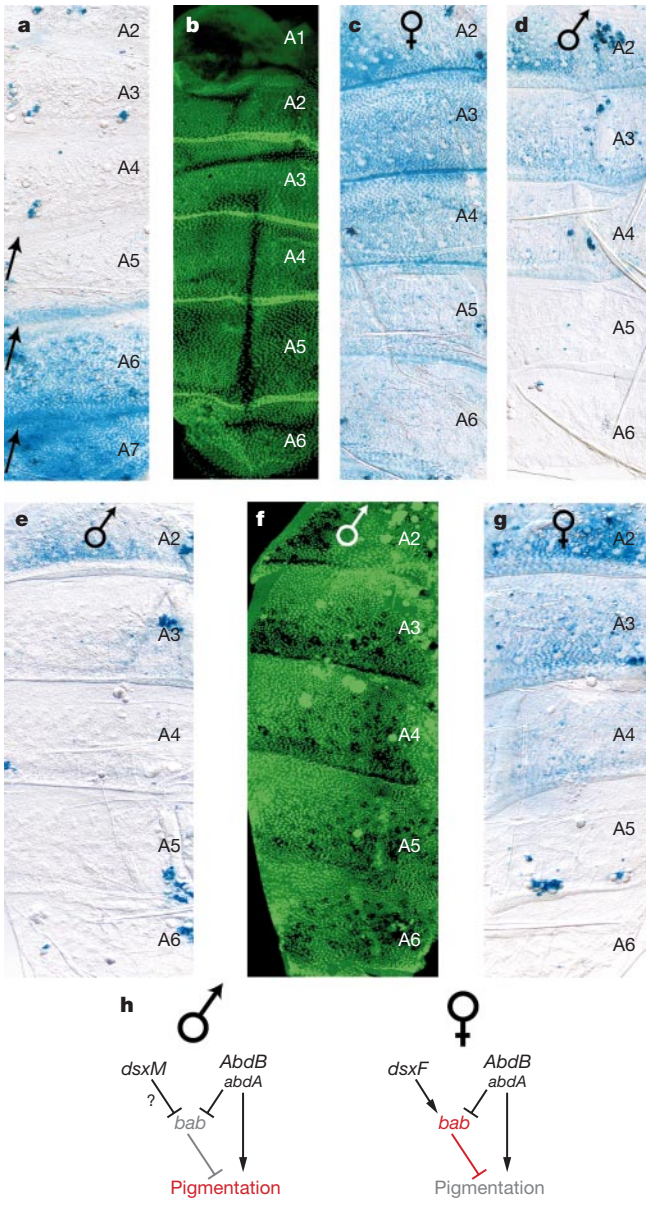

Figure 3 Regulation of *bab* expression by *Abd-B* and *dsx*. All panels show gene expression in the dorsal abdominal epidermis at 40–45 hours after pupariation, shortly before the onset of cuticle differentiation. **a**, *Abd-B-lacZ* female. Segment boundaries are indicated by arrows; *Abd-B* expression is modulated on a parasegmental rather than segmental basis. In A5, expression is only visible in the polyploid bristle cells. **b**, Abd-A is expressed in A2–A7. **c**, **d**, *bab1-lacZ* expression in wild-type female and male, respectively. *bab* expression in A5 and A6 is absent in males (**d**) and downregulated in females (**c**). *bab* expression is first seen at 35 hours after pupariation, shortly before the formation of adult epidermis is completed. *bab* expression is modulated parasegmentally, and is not affected by *hh* or *omb* mutations (not shown). *bab* is also expressed in the ventral abdomen, but at a lower level than in the tergites. Little or no expression is seen in A1. **e**, In $Abd\text{-}B^{Mcp}$ $Abd\text{-}B^{Sab}$/+ males, *bab1-lacZ* is repressed in A3 and A4. **f**, Bab2 expression is derepressed in A5–A7 in a Df(3R)RS4–8/ Df(3R)RS1–98 male. **g**, *bab 1-lacZ* expression in dsx^{1}/dsx^{18} chromosomal females is similar to that in males (**d**). **h**, Control of sexually dimorphic pigmentation of A5 and A6 by *Abd-B, abd-A, dsx* and *bab* (see text). It is unclear whether the same circuit operates in A7, as *bab* expression in females is stronger in A7 than in A6. *bab* expression is also present throughout A8–A10, where it is apparently independent of *Abd-B* and *dsx*.

in repressing male pigmentation. For simplicity, we treat the entire locus as one gene, *bab*, unless noted otherwise.

The expression pattern of *bab* at the pupal stage when the adult epidermis develops reflects its sex- and segment-specific function. In females, *bab* expression is strongest in segments A2 and A3, and progressively weaker in A4, A5 and A6 (Fig. 3c). In males, *bab* expression is considerably weaker than in females in all segments. Most strikingly, it is completely absent from A5 and A6 (Fig. 3d). This pattern of *bab* repression correlates with the presence of sex-specific pigmentation in males, and its absence in females.

To test whether bab^+ is sufficient to repress pigmentation, we ectopically expressed the *bab* genes in the pupal abdomen. Low-level expression of bab^+ results in the loss of male-specific pigmentation, but has no other effects on external morphology (Fig. 2g), indicating that differential regulation of *bab* plays a central role in establishing sexual dimorphism. bab^+ can also repress non-sex-specific pigment stripes when expressed at a higher level (Fig. 2h). This suggests that bab^+ acts as a general repressor of pigmentation, but that its effects are overridden by *omb* in the posterior part of each segment. Consistent with this, complete loss of both *bab* genes results in ectopic pigmentation of A2 to A7 in both sexes (Fig. 2i). This phenotype is not caused by expansion of *Abd-B* expression, which appears normal in these mutants (not shown). In *bab* homozygotes, the intensity of pigmentation is higher in the more posterior segments than in those more anterior (Fig. 2i). This suggests that pigmentation does not develop by default in the absence of *bab,* but is actively promoted by *Abd-B* and *abd-A*.

bab integrates regulatory inputs from *Abd-B* and *dsx*

The sexually dimorphic repression of *bab* in the posterior abdomen suggests that *bab* integrates the homeotic and sex determination regulatory inputs. To test this, we examined *bab* expression in *Abd-B* and *dsx* mutant backgrounds. We find that ectopic expression of *Abd-B* in A3 and A4 eliminates *bab* expression from these segments in males (Fig. 3e), and downregulates it in females (not shown). Conversely, *bab* is derepressed in A5–A7 in the mutants that lack *Abd-B* function in these segments (Fig. 3f). Together, these results indicate that *bab* expression in A5 and A6 is normally repressed by *Abd-B*. The slight downregulation of *bab* in A4 (Fig. 3c and d) suggests that it is also weakly repressed by *abd-A*.

In dsx^- intersexes, *bab* is expressed in a male-like pattern (Fig. 3g), suggesting that *dsxF* upregulates *bab* transcription in females. *Abd-B* and *abd-A* expression is identical in males, females and dsx^- intersexes (not shown), indicating that *bab* is regulated independently by homeotic and sex-determination inputs. $dsx^{Dominant}$ intersexes, which express both male- and female-specific *dsx* products[28], also show male-like expression of *bab* (not shown), indicating that *dsxM* can interfere with *dsxF* function. The two *dsx* isoforms encode transcription factors that bind the same DNA sequence, but have opposite effects on gene expression[29,30]. dsx^- intersexes differ from males in having a small unpigmented region at the anterior-lateral margin of A5 (refs 19, 23; Fig. 2d), suggesting that *dsxM* may have a slight negative influence on *bab* expression.

Our results suggest that bab^+ regulates sexually dimorphic pigmentation by integrating regulatory inputs from the homeotic genes and the sex determination pathway (Fig. 3h). In this regulatory circuit, bab^+ acts as a general repressor of pigmentation, and *Abd-B* and *abd-A* promote pigmentation in both sexes. In addition, *Abd-B,* and to a lesser extent *abd-A*, repress *bab* transcription. In males, this results in the absence of *bab* from A5 and A6, allowing *Abd-B* and *abd-A* to promote pigmentation in these segments. However, in females, *dsxF* prevents *bab* transcription from being completely repressed by the homeotic genes. As a result, *bab* is present in A5 and A6 in females, where it blocks the ability of *Abd-B* and *abd-A* to promote pigmentation. In A2–A4, *abd-A* alone is not sufficient either to repress *bab* or to overcome its inhibitory effect on pigmentation; thus, only the *omb*-dependent striped pigmentation is generated. Because *Abd-B, abd-A* and *dsx* encode transcription factors, they may regulate *bab* expression directly.

Evolution of sexually dimorphic pigmentation

The central role of *bab* as an integrator of homeotic and sex-determination gene inputs suggests that changes in *bab* regulation may have been responsible for the evolution of sexually dimorphic pigmentation. In the subgenus *Sophophora*, male-specific pigmentation is present only in the *melanogaster* species group. Within this group, sexual dimorphism is seen in all species of the *melanogaster* subgroup and the closely related oriental subgroups, whereas the *ananassae* and *montium* subgroups contain both sexually dimorphic and sexually monomorphic species.

We find that in species with male-specific pigmentation of A5 and A6, *bab* expression is absent or strongly downregulated in these segments in males, but not in females (Fig. 4a and b; Fig. 5). Moreover, in the sexually monomorphic species outside the *melanogaster* species group, *bab* expression is identical in both sexes and in all segments from A2 to A7 (Fig. 4c and d; Fig. 5). This correlation suggests that changes in the regulation of *bab* by *Abd-B* and *dsx* played an important role in the origin of sexually dimorphic pigmentation.

Surprisingly, in some species of the *montium* subgroup, such as *D. kikkawai*, *bab* expression in A5 and A6 is downregulated in males despite the absence of sex-specific pigmentation (Fig. 5). This suggests that the function of bab^+ may not be limited to the control of pigmentation. To test whether bab^+ regulates other morphological characters in abdominal segments, we analysed the phenotypes produced by gain and loss of *bab* function in *D. melanogaster*.

Homeotic function of *bab*

In *D. melanogaster*, A5, A6 and A7 differ from the more anterior segments not only in pigmentation, but also in the size and shape of tergites and sternites and in the distribution of bristles and trichomes (Fig. 6a and b). These characteristics, which are especially pronounced in the male, are under the control of *Abd-B*, and the differences among segments are thought to be specified by the different levels of *Abd-B* expression[14–18]. Ectopic expression of *Abd-B* induces posterior characters in anterior segments, while the loss of *Abd-B* function from A5–A7 transforms these segments to a more anterior identity (Fig. 6c).

We find that bab^+ regulates segment shape and bristle and trichome patterns in a manner reciprocal to *Abd-B*. Loss of bab^+ function in females enhances posterior characteristics in A6, A7 and A8 (Fig. 6d). No phenotype is seen in males, consistent with the absence of *bab* expression in posterior segments. Conversely, ectopic expression of *bab* transforms A6 and A7 to a more anterior identity in both males (Fig. 6e) and females (see Supplementary Information). These observations suggest that bab^+ acts as an antagonist of

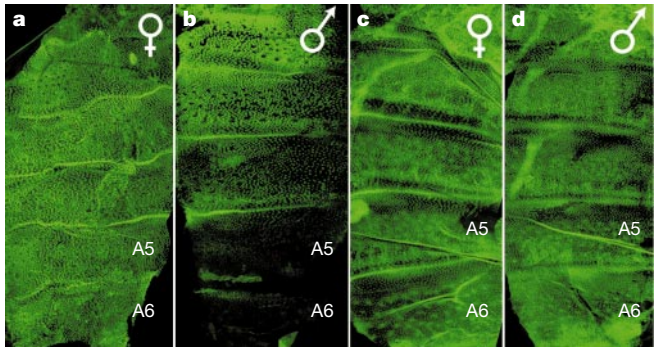

Figure 4 Repression of *bab* correlates with the presence of male-specific pigmentation. Bab2 expression is modulated in the sexually dimorphic species *D. biarmipes* (**a**, female; **b**, male), but not in the sexually monomorphic *D. willistoni* (**c**, female; **d**, male).

Abd-B homeotic function, and that posterior abdominal characters are determined by the balance between *Abd-B* and *bab* activities.

This model predicts that evolutionary changes in *bab* regulation should result in morphological transformation of *Abd-B*-expressing segments. Indeed, we find that the entire suite of characteristics that distinguishes A5 and A6 from the more anterior segments in *D. melanogaster* is of recent evolutionary origin. In *D. willistoni*, *bab* is expressed strongly in A5 and A6 in males (Fig. 4d), whereas *Abd-B* is expressed in the same pattern as in *D. melanogaster* (not shown). As predicted, A5 and A6 are almost identical to the more anterior, non-*Abd-B*-expressing segments in the males of this species (Fig. 6f). In contrast, the *melanogaster* species group shows great diversity of bristle and trichome patterns in posterior abdominal segments (Table 1). The two main lineages within this group show different patterns of evolution. In the clade composed of the *melanogaster* and oriental subgroups, male-specific pigmentation and bristle and trichome patterns have evolved in a concerted fashion (Table 1, group A). However, in the *ananassae* + *montium* lineage, these characteristics vary independently of each other, and sexually dimorphic bristle and trichome patterns are sometimes observed in species that do not show visible modulation of *bab* expression (Table 1, group B, compare with Fig. 5). This suggests that evolutionary changes have occurred not only in *bab* regulation, but also in the target genes of *bab* and in other genes regulated by *Abd-B* and *dsx*. We also note that suppression of A7 development in males has occurred earlier in evolution than visible modulation of *bab* expression, despite the ability of *bab* to override this suppression (Fig. 6e).

Sexually dimorphic pigmentation affects mating preferences

The rapid evolution of sexually dimorphic pigmentation and segment morphology may have been driven by sexual selection. We therefore tested whether male-specific pigmentation confers a competitive advantage in *D. melanogaster* males. Surprisingly, we find that UAS-*bab2*$^{4-66}$ males, which lack male-specific pigmentation but are otherwise normal (Fig. 2g), enjoy the same mating

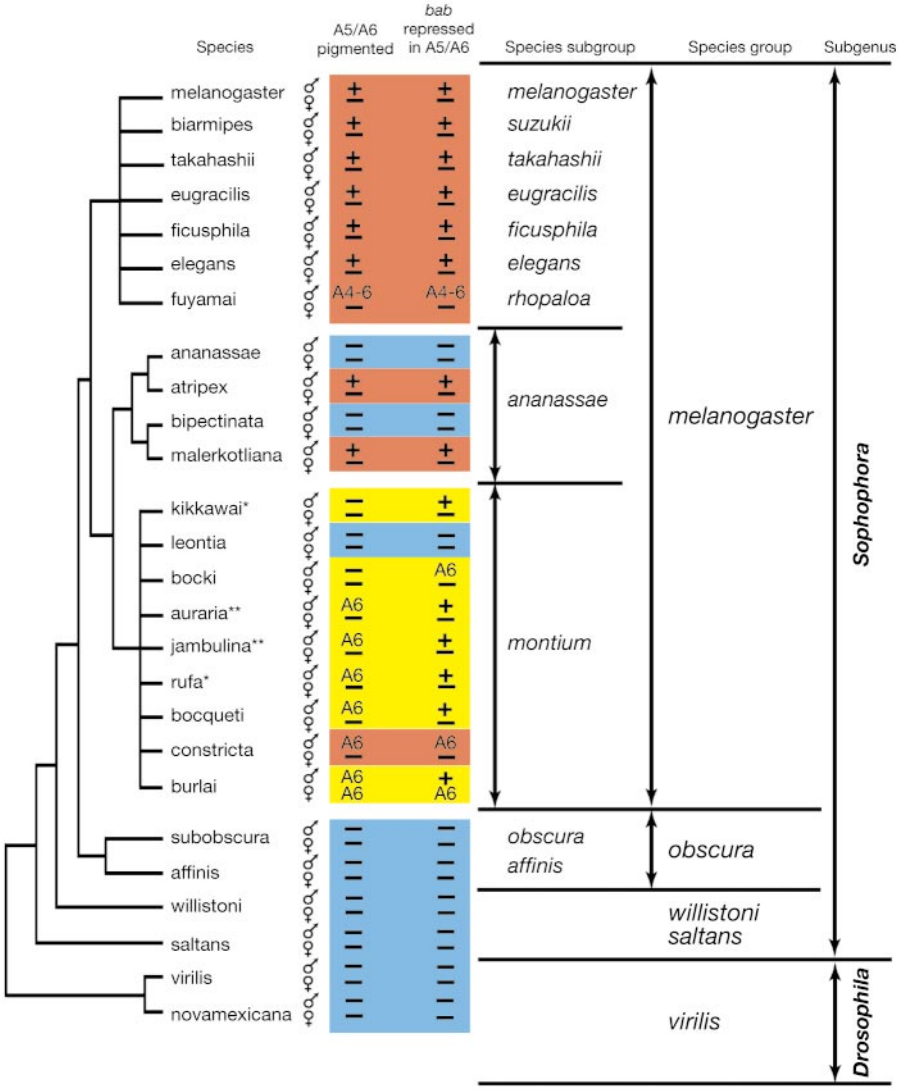

Figure 5 Modulation of *bab* expression correlates with sexual dimorphism. Phylogenetic hypothesis is based on information from various sources[43–46]. The divergence time between *melanogaster* and *obscura* species groups has been estimated at 25 million years[47]. A6 pigmentation is variable in the females of *D. kikkawai* and *D. rufa* (asterisk), and in both females and males of *D. auraria* and *D. jambulina* (double asterisk). Bab2 is repressed in A5 and A6 in the males of sexually dimorphic species (red), but not in the sexually monomorphic species (blue). Exceptions to this rule are found among species of the *montium* subgroup, in which Bab2 is downregulated in the absence of male-specific pigmentation (yellow). Expression was only analysed in the dorsal abdomen. We cannot rule out that species listed as having unmodulated *bab* expression do in fact have subtle modulation that is below the resolution of our methods. For instance, a twofold reduction in the dosage of *bab*, which causes a dramatic pigmentation phenotype in the females of *D. melanogaster* (Fig. 2e), is not detectable by antibody staining. For the same technical reason, quantitative differences between sexes and species were not studied.

success as wild-type males (27 versus 32 matings; $\chi^2 = 0.42$; $P > 0.05$). Thus, although male pigmentation may have been important in the past, it appears to have little or no effect on female mating preferences in extant *D. melanogaster*.

However, we find that *D. melanogaster* males discriminate strongly against heterozygous *bab* females, which have ectopic male-specific pigmentation but are otherwise normal (Fig. 2e), compared with females with lightly pigmented A5 and A6 (23 versus 105 matings; $\chi^2 = 52.53$; $P < 0.001$). Importantly, *white* mutant males, which are visually impaired, mate equally with *bab*/+ and lightly pigmented females (34 versus 39 matings; $\chi^2 = 0.34$; $P > 0.05$), suggesting that discrimination against *bab* heterozygous females is due to their pigmentation. These results suggest that female pigmentation is important in determining their attractiveness to males, and that the absence of male-specific pigmentation in females may be maintained by sexual selection.

A model of evolution of a genetic circuit under sexual selection

Our findings indicate that changes in *bab* regulation have played an important part in the evolution of abdominal segment morphology (Fig. 6g). The presence of *bab* expression in all *Drosophila* species examined suggests that its roles in antagonizing the homeotic function of *Abd-B* and repressing pigmentation are ancestral. However, in the ancestral condition, *bab* expression was independent of *Abd-B* and *dsx*, resulting in sexually monomorphic pigmentation and segment morphology. In the *melanogaster* species group, *bab* evolved to be under the control of *Abd-B* and *dsx*. This eliminated *bab* from *Abd-B*-expressing segments in the male and resulted in a major transformation of male segment morphology. Subsequent diversification of pigmentation, bristle and trichome patterns was probably driven both by the fine-tuning of *bab* regulation and by changes in the downstream targets of *bab* and *Abd-B*.

Two features of this genetic circuit make it highly plastic and evolvable. First, the adult phenotype is sensitive to quantitative changes in *bab* expression. Second, the level of *bab* expression is determined by the balance between *Abd-B* and *dsxF* inputs. If *bab* is regulated directly by *Abd-B* and *dsx*, then the evolution of sexually dimorphic pigmentation and segment morphology may ultimately be traced to the acquisition and modification of binding sites for the Abd-B and Dsx proteins in the *cis*-regulatory region of *bab*. Thus, even a subtle molecular change could be expressed phenotypically and become subject to selection.

This evolutionary model is further supported by the presence of intraspecific genetic variation in sexually dimorphic pigmentation in many extant species[31,32]. In at least one case, there is strong

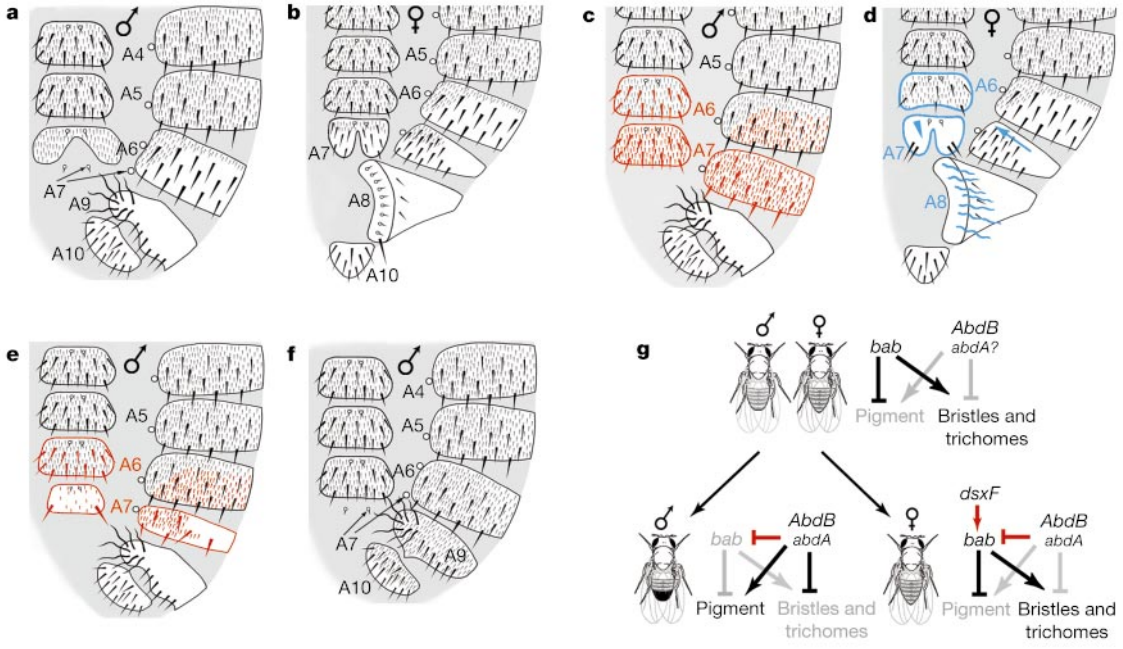

Figure 6 The homeotic function of *bab*. Photomicrographs of the cuticles are available in the Supplementary Information. **a**, Wild-type male of *D. melanogaster*. A7 is greatly reduced and lacks both tergite and sternite. In addition, the A6 sternite lacks bristles and has a unique horseshoe-like shape, and most of the A6 tergite is devoid of the small hairs (trichomes) that cover the more anterior segments. Male genitalia develop from A9, whereas A8 development is repressed. **b**, In a wild-type female, A7 sternite and A7 and A6 tergites are only partly covered by trichomes; the A7 sternite also has a distinctive shape. Female genitalia develop from A8, whereas A9 development is repressed. **c**, In Df(3R)RS4–8/Df(3R)RS1–98 males, A5–A7 are transformed towards A4 identity[18] (indicated in red). The presence of sternite and tergite in A7 is restored, A6 and A7 tergites are completely covered by trichomes, and A6 and A7 sternites are rectangular and carry bristles. **d**, In bab^{Ar07}/ bab^{Ar07} females, expression of posterior morphological characters is increased (blue). Trichomes are absent from the A7 tergite and sternite, and the number of bristles on A6 and A7 sternites is reduced. The A6 sternite also acquires a shape reminiscent of the A6 in the male, or A7 in the female. A weaker phenotype is seen in bab^{Ar07}/+ (not shown). The thorn bristles normally present on the vaginal plates of A8 (**b**) are replaced by long, wavy bristles typical of the male genital arch (A9). bab^{-} females are nevertheless distinguishable from males in the morphology of A6 and particularly A7, indicating that *dsxM* and/or *dsxF* regulate additional target genes in the abdomen. **e**, Ubiquitous *bab* expression in C765-GAL4/UAS-$bab2^{4-51}$ males transforms A6 and A7 to a more anterior identity (red). The presence of tergite and sternite in A7 is restored, A6 sternite acquires a rectangular shape and a number of bristles, and the entire A6 tergite is covered by trichomes. A similar phenotype is caused by misexpression of *bab1* (not shown). The phenotype is enhanced by *abd-A Abd-B* deficiencies, and partly suppressed by *abd-A Abd-B* duplications (not shown). Ectopic *bab* has no effect in the genitalia and analia of either sex (segments A8–A10). The homeotic function of *bab* is apparently limited to the adult stage, because ectopic *bab* expression has no effect in the larval cuticle (not shown). **f**, In *D. willistoni* males, the A6 sternite is rectangular and carries numerous bristles, and the A6 tergite is covered by dense trichomes. We note the similarity to **c** and **e**. **g**, The role of *bab* in the evolution of sexually dimorphic segment morphology. We suggest that sexual dimorphism evolved through the acquisition of two new regulatory interactions, shown in red. The number of genetic steps involved in this transition is unclear. In the *ananassae* and *montium* subgroups, *bab* regulation may differ strongly between closely related species (Fig. 5), indicating that the new genetic circuit retained considerable plasticity.

Table 1 Male-specific characters in two lineages of *melanogaster* species group*

	A5/A6 pigmentation (A6 only in *montium* subgroup)	Loss of A6 tergite trichomes	Loss of A6 sternite bristles
Species			
A			
D. melanogaster	+	+	+
D. biarmipes	+	+	+
D. takahashii	+	+	+
D. eugracilis	+	+	+
D. ficusphila	+	+	+
D. elegans	+	–	+
D. fuyamai	+	+	+
B			
D. ananassae	–	–	+
D. atripex	+	–	+
D. leontia	–	–	+
D. bocki	–	+	+
D. auraria	+/–†	+	–
D. rufa	+	+	–
D. burlai	+	+	+
D. nikananu	–	+	+

* Photomicrographs of some species are available in the Supplementary Information.
† A6 pigmentation is variable in *D. auraria* males.

evidence that allelic differences at the *bab* locus contribute to this variation. Females found in natural populations of *D. melanogaster* vary widely in the extent of A6 pigmentation, ranging from near-zero to 100% (ref. 25). The locus with the largest effect on this variation has been mapped[25]—to the exact position of *bab*. These observations suggest that sexually dimorphic pigmentation evolved through fixation of intraspecific genetic variants at the *bab* locus.

Fixation of new *bab* alleles was probably driven initially by "runaway" sexual selection. In this case, a slight female preference for a weakly pronounced male character would initiate a positive feedback loop that would rapidly increase both the expression of the male character and the female preference for it[33]. This self-reinforcing mechanism can drive rapid character divergence and create new species through sexual isolation[34–37]. Male-specific pigmentation could evolve by this mechanism, with increasingly discriminating females selecting for increasingly dark males. However, once fixed, sexual characteristics can lose their significance as they are overtaken by newly evolving signals and as females become habituated and "resistant" to old characters[38]. This may explain our finding that male pigmentation has no effect on mating success in extant *D. melanogaster*.

Whereas the runaway model explains the evolution of male sexual characters, it does not account for the absence of these characters in females, that is, sexual dimorphism. However, sexual dimorphism can be produced effectively by counter-selection against male-specific traits in females[33]. Consistent with this, we find that *D. melanogaster* males discriminate against females that have male-like pigmentation. In most *Drosophila* species, including *D. melanogaster*, males seek out females at feeding sites and attempt to court as many as possible. Courting other males is not only disadvantageous in competition for females, but may also carry a direct cost[39]. Thus, males are probably selected for an ability to avoid courting other males, and pigmentation may be used to identify females at a distance.

The evolution of *bab* regulation offers a tractable model of how selection creates new morphological characters through changes in DNA sequence. Analysis of the *cis*-regulatory elements of *bab* in sexually dimorphic and monomorphic species will help to clarify the molecular basis of morphological divergence between these taxa. □

Methods

Anterior is upward in all figures. Adult abdomens were cut along the dorsal midline and mounted flat so that ventral cuticle is on the left and dorsal cuticle is on the right. Pupal dissections, X-Gal staining[40] and antibody staining[4] were performed as described.

Drosophila strains and reagents

Abd-B^{Mcp} *Abd-B*^{Sab} is a double gain-of-function mutation that results in ectopic *Abd-B* expression in A3 and A4 (ref. 15). Df(3R)RS4–8 removes the *cis*-regulatory elements responsible for *Abd-B* expression in A5–A7; in Df(3R)RS4–8/Df(3R)RS1–98 heterozygotes, these segments lack *Abd-B* function[18]. *Abd-B-lacZ* enhancer trap HCJ199 (ref. 41) reproduces the pattern of Abd-B protein expression (not shown). Abd-A protein expression was detected by monoclonal antibody Dmabd-A.1 (ref. 42).

bab^{Ar07} (F. Laski, unpublished work) is a small deletion that inactivates both *bab* genes. UAS-*bab2*$^{4–66}$ (D. Godt, unpublished work) is inserted into the 5′ regulatory region of the ecdyson-inducible gene *ftz-F1* (data not shown). The phenotype produced by this line in the absence of a GAL4 driver is presumably caused by low-level expression of *bab2* driven by *ftz-F1* regulatory elements during metamorphosis. In the presence of GAL4, this line generates phenotypes similar to other UAS-*bab* insertions, including UAS-*bab*$^{4–51}$. *pnr*-GAL4 drives gene expression in a stripe along the dorsal midline in all abdominal segments. *bab1-lacZ* enhancer trap[26] reproduces the expression of both *bab* genes. Bab1 and Bab2 proteins, detected by specific antibodies (F. Laski, unpublished work), are expressed in similar patterns in all species examined (*melanogaster*, *virilis*, *willistoni*, *ananassae* and *eugracilis*) (data not shown).

Mating choice experiments

Virgin males and females, 3–5 days old, were placed together in a 95 × 24mm glass vial, and matings were observed visually. For male-choice experiments, a single male was combined with two females of different genotypes. For female-choice experiments, a single female was combined with two males of different genotypes. Only matings that occurred in the first 30 min were counted; the number of unmated groups was also recorded. The *bab* genotypes used were *bab*^{Ar07}/+, *bab*^{Ar07}/TM3, *bab*^{PG}/*bab-lacZ* and Df(3L)Ar12–1/+. Strains in which females have lightly pigmented A6 were isolated from various laboratory stocks, and were not standard wild-type strains. For some experiments, *bab*^{Ar07} was introgressed into a 'light' strain for 3–4 generations. Oregon-R strain was used as a source of wild-type males. For the most part, identical results were obtained with different *bab* alleles and wild-type strains, as well as following the introgression of *bab*^{Ar07} into a lightly pigmented strain, suggesting that mating discrimination is not due to differences in genetic backgrounds.

In experiments with one 'light' strain, wild-type males mated equally with *bab*^{Ar07}/+ and lightly pigmented females (18 versus 22 matings; $\chi^2 = 0.4$; $P > 0.05$). In these experiments, males courted both types of females repeatedly; however, courtship was quickly aborted in most cases, and nearly 50% of males did not mate within 1 hour. The reasons for this behaviour are unclear.

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