Phenotypic Plasticity in *Drosophila*

Pigmentation Caused by Temperature Sensitivity of a Chromatin Regulator Network

Jean-Michel Gibert, Frédérique Peronnet, Christian Schlötterer

1 Institut für Tierzucht und Genetik, Veterinärmedizinische Universität Wien, Vienna, Austria, 2 Université Pierre et Marie Curie-Paris, UMR7622-Biologie du Développement, Centre National de la Recherche Scientifique, Paris, France, 3 Institut für Ökologie, Universität Innsbruck, Innsbruck, Austria

Phenotypic plasticity is the ability of a genotype to produce contrasting phenotypes in different environments. Although many examples have been described, the responsible mechanisms are poorly understood. In particular, it is not clear how phenotypic plasticity is related to buffering, the maintenance of a constant phenotype against genetic or environmental variation. We investigate here the genetic basis of a particularly well described plastic phenotype: the abdominal pigmentation in female *Drosophila melanogaster*. Cold temperature induces a dark pigmentation, in particular in posterior segments, while higher temperature has the opposite effect. We show that the homeotic gene *Abdominal-B* (*Abd-B*) has a major role in the plasticity of pigmentation in the abdomen. *Abd-B* plays opposite roles on melanin production through the regulation of several pigmentation enzymes. This makes the control of pigmentation very unstable in the posterior abdomen, and we show that the relative spatio-temporal expression of limiting pigmentation enzymes in this region of the body is thermosensitive. Temperature acts on melanin production by modulating a chromatin regulator network, interacting genetically with the transcription factor *bric-à-brac* (*bab*), a target of *Abd-B* and *Hsp83*, encoding the chaperone Hsp90. Genetic disruption of this chromatin regulator network increases the effect of temperature and the instability of the pigmentation pattern in the posterior abdomen. Colocalizations on polytene chromosomes suggest that BAB and these chromatin regulators cooperate in the regulation of many targets, including several pigmentation enzymes. We show that they are also involved in sex comb development in males and that genetic destabilization of this network is also strongly modulated by temperature for this phenotype. Thus, we propose that phenotypic plasticity of pigmentation is a side effect reflecting a global impact of temperature on epigenetic mechanisms. Furthermore, the thermosensitivity of this network may be related to the high evolvability of several secondary sexual characters in the genus *Drosophila*.

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**Introduction**

Phenotypic plasticity and buffering are concepts describing the phenotypic outcome of genotype-environment interactions. Phenotypic plasticity is the ability of a given genotype to produce different phenotypes in different environments [1]. It has been the subject of increasing interest as it is involved in adaptation and evolution [1–7]. Buffering, or canalization, is the ability of an organism to maintain a stable phenotype despite genetic variation or environmental fluctuations [8]. Buffering can be challenged by environmental stress, such as extreme temperatures. Thus, the question arises whether the plasticity of a particular phenotype is a specifically targeted reaction of the organism to changes in the environment or whether it is a side effect, reflecting a global process at the level of the transcriptome/proteome, but visible for weakly buffered phenotypes. To answer this question, we investigated the genetic basis of a particularly well described trait subject to phenotypic plasticity: the abdominal pigmentation of female *Drosophila melanogaster*, which strongly depends on the temperature conditions during development [9,10]. In the posterior abdomen, the differences of pigmentation between females grown at 20 °C and 29 °C are comparable to the phenotypic effect of mutations in major structural or developmental regulatory genes. The extreme plasticity of this phenotype makes it a particularly suitable model to dissect the responsible mechanisms. Within the last ten years, key studies have identified structural and developmental regulatory genes playing major roles in abdominal pigmentation patterning [11–16]. Because these studies focused on genetic factors, they were performed under standard temperature conditions [11,13–17]. Following a classical developmental genetics approach, we use mutations in key regulatory or structural genes to destabilize the underlying genetic networks and analyze how they interact with temperature.

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**Abbreviations:** DDC, dopa decarboxylase; TH, tyrosine hydroxylase

* To whom correspondence should be addressed. E-mail: jmg@i122server.vu-wien.ac.at
Author Summary

The phenotype of an individual is not fully controlled by its genes. Environmental conditions (food, light, temperature, pathogens, etc.) can also contribute to phenotypic variation. This phenomenon is called phenotypic plasticity. We investigate here the genetic basis of the phenotypic plasticity of pigmentation in the fruit fly Drosophila melanogaster. Drosophila pigmentation is strongly modulated by temperature, in particular in the posterior abdominal segments of females. The development of these segments is controlled by the homeotic gene Abdominal-B (Abd-B). Abd-B sensitizes pigmentation patterning in this region of the body by repressing several crucial pigmentation enzymes. It makes the regulation of their spatio-temporal expression in the posterior abdomen particularly sensitive to temperature variation. We show that temperature modulates the mechanisms regulating the dynamic structure of the chromosomes. Chromosomal domains can be compacted and transcriptionally silent, or opened and transcriptionally active. Temperature interacts with a network of chromatin regulators and affects not only the regulation of pigmentation enzymes but several traits under the control of this network. Thus, we conclude that the phenotypic plasticity of female abdominal pigmentation in Drosophila is a visible consequence for a particularly sensitive phenotype, of a general effect of temperature on the regulation of chromosome architecture.

Results

Spatial Restriction of Pigmentation Plasticity: Abd-B Sensitizes Pigmentation Patterning to Temperature in the Posterior Abdomen

Pigmentation is sexually dimorphic in D. melanogaster. In males, the abdominal tergites 5 and 6 are black and maintain this pigmentation at all temperatures. In contrast, the posterior abdominal pigmentation in females is highly polymorphic and plastic [18,19]. Figure 1A shows the abdominal pigmentation phenotypes of females from three different wild-type genotypes grown at different temperatures. At a given temperature, the extent of the dark region of the segments in females can differ dramatically between Drosophila lines, showing strong genetic basis [18,19]. NO1 and Samarkand are outliers and most lines have a pattern similar to that of BV1, comparable to the patterns described previously through pigmentation score [10]. Differences in plasticity are observed within each segment along the antero-posterior axis [10] and along the dorso-ventral axis (Figure 1A). This is extreme for A7, which can shift from completely black at 20°C to completely yellowish at 29°C. In addition, the transition border between the yellowish and the dark region of the tergites is not smooth but variegated (Figure 1B), implying that the control of pigmentation is not robust.

The spatial restriction of the phenotypic plasticity of pigmentation suggests the involvement of developmental regulatory genes. The morphology of abdominal tergites A5, A6, and A7 is specified by the posterior homeotic gene Abdominal-B (Abd-B) [20]. In tergites, Abd-B expression is low in A5, intermediate in A6, and high in A7 [21]. Thus, the increasing plasticity observed in the abdomen along the antero-posterior axis [10] perfectly correlates with the expression level of Abd-B. We used the Transabdominal mutation [17] to test whether ectopic expression of this gene in another body region is sufficient to generate a plastic pigmentation pattern. This mutation is a chromosomal rearrangement that fused the regulatory region of the stripe gene to the Abd-B locus [17,22]. It induces an ectopic expression of Abd-B on the thorax at the flight muscle attachment sites. This phenotype was previously described as sexually dimorphic, inducing melanin production in the whole sites of ectopic expression in males and in only restricted areas in females [17]. The pattern is indeed sexually dimorphic, but it is also extremely plastic (Figure 2). Remarkably, in females, the regions that are not brown at the sites of ectopic Abd-B expression show a very strong reduction in the production of yellowish pigments (Figure 2G–2L). This indicates that Abd-B plays opposite roles in melanin production. It either increases melanin production or represses the production of all pigments. Furthermore, these two roles are extremely thermostensitive. The increase of melanin production is much higher at low temperature, whereas the decrease in pigment production is much stronger at high temperature. These two roles of Abd-B are concomitantly observed within the same spot of ectopic expression, which suggests that they are influenced by other developmental pathways.

In order to quantify the effects of Abd-B and temperature on pigmentation, we tested how modifications of Abd-B expression level interact with temperature in the development of the pigmentation pattern. We varied the copy number of Abd-B using a deficiency of Abd-B (Df(3R)RS-I–98) and a duplication of Abd-B (Dp-P5). Both mutations are carried in the same stock, which reduces background effects as much as possible. We found that high temperature decreases melanin production in all genotypes, but the effects of Abd-B level differed in A6 and A7 and along the dorso-ventral axis within A6 (Figure S1A–S1I). Thus, we quantified the melanin production along the antero-posterior axis in the lateral, median, and dorsal region of A6, in the lateral region of A7, and along the dorso-ventral axis in A7 (Figure S2). Temperature, Abd-B as well as the Abd-Bxtemperature interaction, strongly influenced pigmentation in each of these regions (p < 0.001 for all), except the dorsal midline (Table S1). These effects explained a large proportion of the total variation in abdomen pigmentation. The Abd-Bxtemperature interactions are particularly striking in the lateral regions of A6 and A7 and in the median region of A6 (Figure 3). In line with previous studies [13], Abd-B strongly increases melanin production in the lateral region of A6 under all temperature regimes (Figure 3A). The Abd-Bxtemperature interaction in the lateral region of A6 was mainly attributable to the pronounced reduction in pigmentation at high temperatures when the expression of Abd-B is low (Figure 3A). There was also a significant interaction between Abd-B and temperature in the lateral region of A7 (Figure 3C). However, in contrast to the lateral region of A6, Abd-B represses melanin production in the lateral region of A7. The opposite roles of Abd-B on melanin production are best illustrated by the very pronounced Abd-Bxtemperature interaction in the median region of A6. At low temperature, high Abd-B levels increase melanin production, whereas at high temperature they reduce melanin production (Figure 3B).

Based on these data, we conclude that Abd-B has two opposite roles on melanin production in females and can either increase or decrease melanin production. This makes the balance between melanin production and repression very unstable in the posterior abdomen, generating phenotypic plasticity in pigmentation. Indeed, this balance is very
sensitive to temperature and is most pronounced in A7 showing the highest Abd-B level.

**Temperature Modulates the Spatio-Temporal Expression of Limiting Pigmentation Enzymes in the Posterior Abdomen**

*Abd-B* is a developmental regulatory gene encoding a homeodomain transcription factor [23]. Its opposite roles on melanin production must be ultimately mediated by pigmentation enzymes. Indeed, pigment precursors move only a few cell diameters; thus, the spatial restriction of some of the enzymes synthesizing them is directly responsible for the pigmentation pattern observed in the adult [14,24,25]. A consensus model of pigment synthesis pathway is discussed in Text S1. Two classes of enzymes can be distinguished. Enzymes of the first class such as the tyrosine hydroxylase (TH) or the dopa decarboxylase (DDC) are required for the production of pigment precursors involved in the synthesis of all pigments. Enzymes of the second class, such as Ebony, Yellow, or Tan, are involved in the switch between the

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**Figure 1. The Pattern of Abdominal Pigmentation Plasticity in Wild-Type Females**

(A) Abdominal phenotypes of females from the inbred wild-type lines NO1, BV1, and Samarkand grown at 20 °C, 25 °C, and 29 °C. The drawings on the right summarize the plasticity of the different regions of the body according to the color code. A1–A7, abdominal segment number; L, lateral region; D, dorsal region; SAM, Samarkand.

(B) The pigmentation in wild-type females shows a variegated pattern. (1) Left and right 7th hemitergites of a BV1 female grown at 25 °C. The pigmentation is not perfectly symmetrical. White patches are visible on one side (arrows) where dark pigmentation is observed on the other side. The dark pigmentation follows the insertion of the small bristles in the inner region of the tergite (arrowheads). (2 and 3) Shows the 6th hemitergites of two NO1 females grown at 25 °C. The pigmentation patterns are very similar but not perfectly identical. The limit between the dark and yellowish regions of the tergite is not smooth but variegated. Yellowish patches (arrows) at the base of some bristles are surrounded by dark pigmentation (3, arrowheads).

![Figure 1](https://example.com/figure1.png)

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production of yellowish (NBAD sclerotin) or black-brown (dopa-melanin and dopamine-melanin) pigments [14, 26].

The strong reduction in the production of all pigment observed in regions expressing Abd-B on the thorax of Tab+/ females suggests that Abd-B represses melanin production through the downregulation of one or several enzymes of the first class. The strong production of melanin observed at low temperature in the posterior abdomen and in some of the first class. The strong production of melanin observed at low temperature in the posterior abdomen and in some of the first class. Mutations in genes encoding enzymes of the first class are homozygously lethal but loss-of-function mutations for enzymes of the second class are homozygously viable and can be used to identify the target(s) of Abd-B involved in plasticity. We postulated that if phenotypic plasticity of pigmentation is caused by temperature-dependent activity or regulation of a particular pigmentation enzyme, loss-of-function mutations in the gene encoding this enzyme should generate a nonplastic pigmentation phenotype. We used mutations in yellow (y1), tan (t1), and ebony (e1) (Figure S3). We observed that the plasticity of abdominal pigmentation is still visible in females mutant for y (Figure S3D–S3F), which lack black melanin but still have brown melanin. In t mutants, melanin is produced in males and females in the posterior abdomen at 20 °C, but is strongly reduced at higher temperatures in both sexes (Figure S3G–S3I and S3J). In contrast, e flies remain very dark at high temperature and show very limited plasticity of pigmentation (Figure S3J–S3L). Thus, a functional e gene is required for the plasticity of pigmentation. In flies mutant for t, which antagonizes e [15], the role of e is magnified. This suggests that the system responsible for plasticity in A5 or A6 also exists partly in males, but that it is normally hidden by the activity of Tan. The production of melanin in t mutants requires the repression or the strong downregulation of e, as even the gain-of-function of y cannot induce melanin production in the presence of Ebony [14]. Thus, the pigmentation pattern of t mutants at different temperatures implies that e is differentially regulated at different temperatures, relative to the production of melanin precursors, and that a major temperature-induced regulatory switch occurs between 20 °C and 25 °C.

We thus investigated the expression of the genes encoding Ebony and the two enzymes of the first class, TH and ddc. In order to visualize the expression of these enzymes, we stained pharate adults dissected out of their pupal case. We first investigated the expression pattern of e, ddc, and TH at 25 °C using e-LacZ, ddc-LacZ, and UAS-LacZ, TH-Gal4 flies, respectively. We observed that e, ddc, and TH are highly expressed in the pattern of the thoracic trident (Figure S4). The trident is a cryptic pattern fully visible in e mutants, y; e double-mutants (Figure S4B and S4C) [14], or when flies are grown at extreme temperatures [27] (Figure 2A). A similar pattern was described with an antibody against Ebony [14]. This suggests that the coexpression of e with TH and ddc assures that most of the locally produced pigment precursors are normally converted into yellowish NBAD sclerotin by Ebony. In the absence of Ebony, the excess of dopamine is converted into dopamine-melanin [14]. Thus, the melanin pattern in the absence of e is completely correlated to the spatial expression of TH and DDC in the epidermis.

We then looked at the expression of these enzymes in abdominal segments to see how their combined spatio-temporal expression could explain the pigmentation pattern. We observed with the e-LacZ transgene an expression similar to that previously reported using an antibody against Ebony [14]. It starts at the base of the bristles around 90 h after puparium formation (Figure 4A) and then becomes progressively uniform in the epidermal cells of the tergites (Figure 4B and 4C). We observed that the epidermal expression starts in the anterior region of the segment, as a weak antero-posterior gradient is first visible (Figure 4B). e-LacZ is stronger in the anterior region (Figure 4B, arrowhead) than in the posterior region of the segment (Figure 4B, arrow). We observed that the expression of ddc is also very dynamic in the posterior abdomen (unpublished data), but it is even more pronounced for TH, which encodes the first and limiting enzyme in the pigment synthesis pathway. Thus, we focused on TH. In the abdomen, TH expression started at the base of the large bristles on the posterior border of the
segments before complete maturation of the bristles (Figure 4D). Expression at the base of more anterior bristles begins later (Figure 4E). Finally, TH is later expressed in epidermis of the whole tergites (Figure 4F). In the abdomen, it is expressed along an antero-posterior gradient as the expression starts much later in the more posterior segments (Figure 4D–4F). In particular, no strong expression is visible before hatching in A7 (Figure 4F). Thus, the expression of TH is lower in the posterior abdomen where the Abd-B level is the highest. We then looked at the expression of TH on the thorax of Tab/+ pharate females. We observed that it is also very dynamic. The expression is first visible at the base of the bristles located in the region of the trident (Figure 4G); then, a uniform expression is visible in the epidermal cells of the trident (Figure 4H). In the regions of ectopic Abd-B expression, expression starts at the base of the bristle located close to the teeth of the trident (Figure 4H, arrowhead). Later on, it is visible in the epidermal cells of these regions, but the regions that are devoid of pigments in Tab/+ flies show a much reduced staining (Figure 4I, arrows). Thus, the most plastic regions, i.e., the posterior abdomen and the regions of Abd-B ectopic expression in Tab/+ females, express TH very late. Furthermore, regions of ectopic expression of Abd-B devoid of pigments correspond to strongly reduced expression of TH. The delayed pigmentation in the posterior abdomen and the loss of TH expression in the regions of ectopic expression of Abd-B in Tab/+ females suggest that Abd-B represses TH, at least indirectly. There is an obvious difference between e and TH expression at 25°C in the posterior abdomen. In particular, at 25°C, e is already expressed in A7 before hatching, whereas TH is not yet expressed. We assume that TH is expressed in A7, which is pigmented, but this expression probably occurs after hatching. This is likely, as the activity of TH was reported to peak 50 min after hatching [28]. This means that when TH starts to be expressed, e being already expressed, DOPA and dopamine can be used to produce NBAD, the precursor of the yellowish pigment. Because the phenotype of tan mutants suggested that a major regulatory switch occurs between 20°C and 25°C, we analyzed the expression of e and TH in females grown at 20°C. When flies are grown at 20°C, the expression of e just before hatching is much weaker in the posterior abdomen than at 25°C (Figure 4J). Furthermore, at 20°C, TH expression can be observed very clearly before hatching in A7, but is mainly seen in association with bristles in the inner region of the tergite (Figure 4K).

Thus, Abd-B plays opposite roles in melanin production by repressing at least two genes encoding pigmentation enzymes with different roles in melanin production: TH required for the production of all pigments and Ebony required for the production of the yellowish pigment. It makes the expression of these enzymes particularly sensitive to temperature in the posterior abdomen. At low temperature, the stronger repression of e and the reduced repression of TH correlate with the increased melanin production observed in the posterior abdomen and on the thorax of Tab/+ females. In contrast, at higher temperature, the strong repression of TH and its delayed expression correlate with the reduced pigment production observed in the posterior abdomen and on the thorax of Tab/+ females. The effect on expression timing is visible on the pigmentation phenotype of the A7 tergite in limiting conditions, for example, in females with
three doses of Abd-B grown at 25 °C: the melanin remaining is clearly associated with bristles, which mark the first sites of TH expression (Figure 4L).

Temperature Affects a Network of Chromatin Regulators Interacting with the Transcription Factor Bric-à-Brac

How could temperature influence these opposite roles of Abd-B on pigmentation? Abd-B was previously shown to induce melanin production both via the repression of the transcription factor bric-à-brac (bab) and independently of bab [13]. bab, which was shown to repress melanin production, is strongly repressed in males by Abd-B in A5 and A6 [13]. This leads to the melanic pigmentation observed in the posterior abdomen [13]. bab is activated by the female-specific isoform of doublesex (dsxF), which compensates for the repression of
bab by Abd-B, and thus reduces the amount of melanin produced in this part of the abdomen compared to males [13]. In female A7, bab is not repressed by Abd-B [13]. In order to analyze potential interactions between bab and environmental temperature, we used the babAR07 mutation that completely abolishes the expression of the two paralogs bab1 and bab2 and induces a well characterized haploinsufficient melanic phenotype [13,18,29]. We observed that this phenotype is fully visible at 25 °C in A6, but is less obvious at other temperatures compared to wild-type (Figure S1J–S1L). Multivariate analysis of the effect of bab and temperature on melanin production (Table S2) revealed a very strong effect of bab and bab×temperature interaction in the lateral and median region of A6 and along the dorsoventral axis of A7 (p < 0.001 for all, Figure 5A and 5B; Table S2). No significant effect was observed in the lateral region of A7 (Figure 5C). Thus, BAB level is less limiting in wild-type flies in A7, where bab is not repressed by Abd-B, than in A6. The role of bab on melanin production has been described previously [13,29], but these experiments did not reveal whether bab acts mainly by regulating pigmentation enzymes of the first or the second class. To identify the main targets of bab, we overexpressed bab1 in the dorsal domain using the pannier driver as previously described [13], but in an e or in a y background (Figure 6A and 6B). We observed that the production of both melanin and yellowish NBAD sclerotin is strongly repressed by the overexpression of bab1 (Figure S5A and S5B, arrows). It suggests that bab represses an enzyme of the first class. BAB has been reported to physically interact with products of the Broad-Complex [30], a direct regulator of ddc in pharate adults [31]. We used a ddc-lacZ transgene and observed that ddc-lacZ is downregulated in the dorsal domain of flies overexpressing bab1 (Figure S5C, compared to Figure S5D).
SSD, arrows). Thus, the effect of \textit{bab} on melanin production is mediated at least through the repression of \textit{ddc}.

Interestingly, temperature was shown previously to modulate the effect of \textit{bab} loss-of-function on another phenotype: the presence of an ectopic sex comb observed in males on the second tarsal segment of the first leg [32]. The sex comb is a structure made of modified bristles located on the first tarsal segment of the first leg in \textit{Drosophila melanogaster} males. Ectopic sex comb composed of two and three teeth, respectively, can be seen on the second tarsal segment (T2) in the flies grown at 25 and 29 °C (arrows).

(B) Sex comb phenotype of \textit{crm}/+; \textit{bab}AR07/+ of a fly grown at 29 °C. An ectopic sex comb caused by \textit{crm} alone is visible on the first tarsal segment (T1) of the second leg (L2), a typical PcG phenotype as previously reported [35]. A strong synergistic interaction between \textit{bab} and \textit{crm} is revealed by ectopic sex comb teeth, not only on the second (T2) but also on the third (T3) tarsal segment of the first leg (L1). Note that the legs are shortened and that the second tarsal segment is inflated, affecting the ectopic sex comb shape.

(C) Mean and standard errors of sex comb teeth on the second tarsal segment of the first leg in wild-type (green), \textit{bab}AR07/+, \textit{crm}/+ and \textit{crm}/; \textit{bab}AR07/+ (red) males grown at 25 °C.

(D) Mean and standard errors of sex comb teeth on the second tarsal segment in \textit{bab}AR07/+ single heterozygotes and in combinations with the other mutations indicated on the right grown at 20 °C, 25 °C, and 29 °C.

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Figure 6. Chromatin Regulators, Temperature, and Sex Comb Development

The sex comb is a structure made of modified bristles located on the first tarsal segment of the first leg in males. (A) Sex comb phenotype of representative \textit{bab}AR07/corto420 males grown at 20 °C, 25 °C, and 29 °C. The normal sex comb is located on the first leg in the first tarsal segment (T1). The ectopic sex comb composed of two and three teeth, respectively, can be seen on the second tarsal segment (T2) in the flies grown at 25 and 29 °C (arrows).

S5D, arrows). Thus, the effect of \textit{bab} on melanin production is observed also in some chromatin regulator mutants of the Polycomb group (PcG) and Enhancer of Trithorax and Polycomb group (ETP) [35–36]. The Polycomb group (PcG) and the antagonizing Trithorax-group (TrxG) proteins were identified through their role in the regulation of homeotic genes (Hox) [37], but it is now clear that they regulate hundreds of genes [38,39]. The PcG are involved in silencing of Hox genes, whereas the TrxG are involved in their activation. A third class of chromatin regulators has been described, the Enhancers of trithorax and Polycomb (ETP), required for both TrxG and PcG normal functions [40]. Most PcG mutants
induce the formation of ectopic sex combs on the first tarsal segment of the second and third legs caused by ectopic expression of the homeotic gene Sex-comb reduced (Scr) [37,41]. However, ectopic distal sex combs are observed in mutants of only a few PcG or ETP genes [34–36]. This suggests that these different ectopic sex comb phenotypes correspond to the disruption of two distinct processes, and that bab and a subset of chromatin regulators are required for the repression of the distal sex comb. Other data suggest that these genetic interactions probably correspond to physical interactions between BAB and chromatin regulators. The bab locus encodes two closely related transcription factors with a BTB/POZ domain [29]. This interaction domain is present in many chromatin regulators [42] or transcription factors recruiting chromatin regulators [43]. In particular, BAB has been reported to bind to Batman/LolaL, which is part of PcG and TrxG complexes [42]. Interestingly, the activity of chromatin regulators such as members of the Trithorax/Polycrom system are known to be temperature-sensitive [38,44]. Silencing by PcG through characterized regulatory sequences known as Polycomb Response Elements was shown to be stronger at high temperature [38,44,45]. Thus, we hypothesized that the modulation of bab activity by temperature could take place via an effect of temperature on a network of chromatin regulators interacting with BAB.

We used the sex comb phenotype to test for genetic interactions between bab, genes encoding ETP or PcG, and temperature. We observed strong genetic interactions between bab and corto, cramped (crm), batmanlolaL, and Trithorax-like (Trl) that encodes GAGA (Figure 6). Temperature strongly enhances the sex comb phenotype of crm/Y; babAR07/+ males. At 29 °C, they die in their pupal case with sex comb teeth also visible on the third tarsal segment of the first leg (Figure 6B) in a large fraction of the individuals (6/18 observed legs). This phenotype is not observed at lower temperature or in single mutants. In addition, the second tarsal segment is inflated and shortened, which reduces the size of the ectopic sex comb and makes quantification impossible. Therefore, we quantified the crm-bab interaction only at 25 °C using the number of teeth in the ectopic sex comb on the second tarsal segment of the first leg (Figure 6C). Wild-type flies have no sex comb teeth on the second tarsal segment (0 ± 0, n = 16). The crm/Y; babAR07/+ males have many more teeth (6.18 ± 0.25, n = 22) than crm/Y hemizygotes (2.75 ± 0.18, n = 12) and babAR07 heterozygotes (0.18 ± 0.05, n = 36). This strong genetic interaction is shown in Figure 6C. We also quantified the effect of temperature on the interactions between bab and other chromatin regulators (Figure 6A and 6D, Tables S3 and S4). The single heterozygote mutants for corto, ban, or Trl do not show ectopic sex combs. We analyzed how these mutations modify the babAR07/+ phenotype. The genotype and the temperature accounted for 71% of the variance (Table S4). Temperature had a strong effect and increased sex comb teeth number across all genotypes. All heterozygote double mutants were significantly different from babAR07/+ (Tukey post hoc test, p < 0.001), which shows that the effect of bab mutation is strongly enhanced by mutations in corto, ban, lolaL, or Trl. We tested three corto alleles. All alleles showed a similar trend, but the effects were stronger for cortoY and cortoL. In addition, there was a significant genotypetemperature interaction (Table S4) visible in the curves corresponding to babAR07 single mutants and double heterozygotes (Figure 6D).

Given these strong effects, we analyzed the effect of mutations in chromatin regulators on the abdominal pigmentation of babAR07 heterozygote females. All the mutants we looked at had been induced in different backgrounds, so it was not possible to clearly differentiate the effect of the mutation itself from the background. Balancers are frequently used as control when the mutant stock is out-crossed to a wild-type line. However, most of the balancer chromosomes from the mutant stocks carry mutations in pigmentation genes, which is particularly inadequate in our case. Thus, we focused on genes for which we observed very strong phenotypes and interactions, and/or for which we could test different alleles. We observed very strong effects for the three corto alleles. They dominantly induce a reduced pigmentation in A7 at 25 °C. This is extreme for cortoY and cortoL (Figures S6 and S7) and weaker for cortoT218 (Figure S7E). We tested how they would modify the haplo-insufficient pigmentation seen in babAR07 females. We observed a strong temperature-sensitive effect on pigmentation in babAR07/cortoY in A6 with a completely black phenotype at 20 °C, a strong variegation at 25 °C, and a completely white pigmentation at 29 °C (except for the dorsal midline) (Figure S7G–S7I). In A7, the pigmentation was very weak at 25 °C. A similar effect was observed with cortoL (Figure S8) and a visible but weaker effect with cortoT218 (Figure S8). Quantification of melanin production revealed very strong effect for cortoY and strong interactions between corto and temperature and between bab, corto, and temperature (Figure 3A–3C, Table S1). In particular, whereas reducing bab level by half has no significant effect on melanin production in the lateral region of A7, it interacts very strongly with corto for this phenotype (Figure 3C, Table S1). In addition, in the median region of A6, babAR07/cortoY females produce less melanin than wild-type or single heterozygous females, whereas reducing bab level alone has the opposite effect (Figure 3B). This corresponds to the variegated phenotype observed at 25 °C (Figure S7H) and shows that bab and corto work together to increase melanin production in this region of A6. The females homozygous for the crmY allele of the PcG gene crm show an absence of melanin in A7 and a very reduced and variegated pigmentation in A6 (Figure S8A). This is not observed in their heterozygote siblings crm/YM7c (Figure S8B) or when out-crossed to a wild-type stock (Figure S8C). We also quantified how heterozygosity for crmY would modify the pigmentation phenotype of babAR07/+ females. Except for the dorsal midline, we observed strong effect of crm and interaction between crm and bab, betweencrm and temperature, and between crm, bab, and temperature (Table S1).

The genetic interactions between bab and chromatin regulators for abdominal pigmentation and sex comb development, and previously reported physical interaction between BAB and the ETP Batman/Lolal [42], suggest that BAB and chromatin regulators cooperate in the regulation of particular targets. In order to test this hypothesis, we used antibodies against BAB, CRM, and Corto to localize their products on salivary gland polytene chromosomes. We observed many colocalizations on polytene chromosomes of BAB with Corto and BAB with CRM (unpublished data). In particular, we observed clear staining for BAB, Corto, and CRM in the cytological region corresponding to the locus of TTH (65C) (Figure 7). BAB and CRM colocalized in the cytological region of the ddc (57C) (Figure 7G and 7H). We
detected BAB alone in the cytological region of e (93C) (Figure 7E, 7F, 7K, and 7L).

This Chromatin Regulator Network Interacts Genetically with the Chaperone Hsp90

Chaperones, in particular Hsp90, have been shown to buffer against the effect of cryptic genetic variation and environmental stress, in particular against high temperature [46,47]. Recent studies revealed a link between the chaperones and chromatin regulators [48–50], which suggested that the effect of temperature on chromatin regulators might be partly mediated by chaperones. We tested this hypothesis using two different alleles of Hsp83, the gene encoding Hsp90. In females, the allele Hsp83e6D dominantly induced a very low
pigmentation in A7 at 25 °C (Figure S6Q), not observed with the allele Hsp83^{60A} (unpublished data), which had a weaker TrxG-like effect than Hsp83^{60A} [48].

We tested the effect on abdominal pigmentation of the two Hsp83 alleles in bab^{A05} heterozygous females. We observed that Hsp83^{60D} (Figure S6T and S6U), but not Hsp83^{60A} (unpublished data), strongly reduced the pigmentation phenotype of bab^{A05}^/+ at 25 and 29 °C. Quantification of melanin production revealed strong effect of Hsp83^{60D} and a strong interaction with temperature (Table S1). In contrast, interactions between Hsp83^{60D} and bab were only significant in the median region of A6. Significant interactions were observed between bab, Hsp83^{60D}, and temperature in the median region of A6 and along the dorso-ventral axis of A7 (Table S1). We also quantified the effect of Hsp83^{60D} on bab^{A07} heterozygote sex comb phenotype and found that it increased the number of teeth in the ectopic sex comb at 20 °C and 25 °C, but slightly decreases it at 29 °C (Figure 6D, Table S4). Because of the similarity of effects of Hsp83 and corto on the pigmentation phenotypes, we tested for potential genetic interactions between these two genes. We observed extragenic non-complementation (lethality) between Hsp83^{60D} and corto^{402} (observed when crossed in both directions), whereas corto^{402} is viable with Hsp83^{60A}. The Hsp83^{60D}/corto^{420} and Hsp83^{60D}/
corto^{7128} genotype were viable, but strongly enhanced the loss of pigmentation observed in Hsp83^{60D}^+/corto^{420} or, or corto^{7128}^+/ females at 25 °C (Figures S6V–S6X and S7P–S7R). Quantification of melanin production revealed very strong interactions between Hsp83 and corto and between Hsp83, corto, and temperature (Figure 5D–5F; Table S1). In particular, the double heterozygote Hsp83^{60D}/corto^{420} females have an extremely reduced melanin production (Figure 5D–5F) and are the only genotype we analyzed where the pigmentation in A6 at the dorsal midline is affected at 25 and 29 °C. Furthermore, the Hsp83^{60D} allele (Figure S9) also dominantly induced pigmentation defects in males in the A5 segment observed at 25 °C and 29 °C, but not at 20 °C (Figure S9B and S9C). Most importantly, corto alleles strongly increased the pigmentation defects observed in Hsp83^{60D}^+/ males at 25 °C and 29 °C (Figure S9D–S9L). Loss of pigmentation was visible in A6 with both alleles. In contrast, corto^{420}^+/ and corto^{7128}^+/ males had a normal pigmentation in A5 and A6 at all tested temperatures (unpublished data). We observed a similar phenotype in Hsp83^{60D}/bab^{A07} males (Figure S9J–S9L). This suggests that Hsp83, bab, and corto work tightly together to control abdominal pigmentation in both males and females and are much more required at 25 °C and 29 °C than at 20 °C.

**Discussion**

Buffering or canalization describes the ability of individuals of a given species to show a constant phenotype despite genetic variations or environmental fluctuations. Phenotypic plasticity could therefore be interpreted as a weaker buffering of some phenotypes. Chaperones, and in particular Hsp90, have been identified as components of this buffering system and are thought to become limiting under stressful conditions such as high temperature [46,48]. The chaperone Hsp90 was proposed to act as a general evolutionary capacitor by releasing the effect of cryptic genetic variation under stressful environment [46]. However, more recent studies have revealed that the influence of Hsp90 on the variation of particular traits was very limited [51]. This suggests that the ability of chaperones, and Hsp90 in particular, to buffer phenotypic variation is not so general, but might rely on very specific interactions more tightly involved in particular phenotypes. Recent studies revealed a link between chaperones and chromatin regulators [48–50]. In particular, Hsp90 and several TrxG chromatin regulators were shown to buffer the same phenotype [48]. These studies on buffering in flies were based on the penetrance of deleterious phenotypes caused by cryptic genetic variation or known introduced mutations [46,48]. We found that mild modulation of a similar system by environmental temperature is involved in phenotypic plasticity of abdominal pigmentation. The chromatin regulator network we found to be sensitive to environmental temperature interacts genetically with the chaperone Hsp90 and the transcription factor BAB. It contains the PcG gene *crm* and the ETP gene *corto*. We observed very strong genetic interactions between *corto* and Hsp83, the gene encoding *crm* [48,50]. In particular, we observed extragenic noncomplementation between Hsp83^{60D} and *corto^{402}*, whereas the viable trans-heterozygote combinations with the two *corto* alleles induce strong reduction in melanin production in both sexes. This suggests that Hsp90 and Corto are involved in a common process. Hsp90 has been shown to physically interact with histones, to induce chromatin condensation, and to interact with topoisomerase II, which plays a crucial role in chromosome condensation [52,53]. Interestingly, *corto* is also required for the normal condensation of chromosomes during mitosis [33]. Therefore, the interactions between *corto* and *hsp83* in gene regulation might be linked to a general role and common involvement of these genes in chromatin condensation.

The pigmentation in the posterior abdomen is particularly sensitive to environmental temperature because it is sensitized by the input of the homeotic gene *Abd-B*. *Abd-B* plays opposite roles in melanin production. The positive role of *Abd-B* in melanin production in females has already been described [13]. It is linked to the establishment of the sexually dimorphic pattern of pigmentation, and, for this role, *Abd-B* works antagonistically with *bab* by repressing it in A6 and A5 [13]. The role of *Abd-B* in repressing melanin production has not been described previously. It is very strong in A7 and is probably linked to the very peculiar morphology of this segment in females. In A7, *bab* is not repressed by *Abd-B*, and both genes work together not only to repress melanin production, but also to control some aspects of the particular development of this segment such as the absence of fusion of the tergites at the dorsal midline [29,54]. *Abd-B* plays these opposite roles in melanin production by repressing several pigmentation enzymes such as *TH* and *Ebony*. These enzymes start to be expressed at the end of pupal development [14,28]. Modulation of the regulation of these enzymes by temperature induces a local difference in their relative timing of expression in the abdominal epidermis. The effect is particularly visible in A7, which exhibits the highest *Abd-B* level. Studies in *Drosophila* wing have shown that pigment precursors can also be provided by the hemolymph [55]. Hence, it is possible that a change in the general level of pigment precursors in the hemolymph might contribute partly to the phenotypic plasticity of abdominal pigmentation. However, the diffusion of pigment precursors from the hemolymph does not seem to play an important role in the
pigmentation of the body: recent studies in lepidopterans showed that the local production of DOPA and dopamine by TH and DDC in the epidermis are major components of the pigmentation pattern [56]. In Drosophila abdomen, epidermal clones of cells mutant for TH or DDC are albino [55], which shows that pigment precursors potentially available in the hemolymph cannot contribute significantly to pigment production in the body epidermis. In addition, in the thorax, the pigmentation patterns visible in e and y, e mutants perfectly correlate with the epidermal expression of TH and DDC, the enzymes providing pigment precursors (Figure S4). Furthermore, we clearly show that the spatial restriction of plasticity is strongly conditioned by Abdominal-B expression and the repression of pigment precursor production in the thoracic epidermis in Transabdominal mutants (Figure 2).

Thus, we conclude that the modulation of the relative temporal expression of TH and ebony by temperature in the epidermis of the posterior abdomen is responsible for the phenotypic plasticity of female abdominal pigmentation. Mutations in corto, crm, hsp83, and bab enhance the effect of temperature on melanin production in the posterior abdomen. The colocalization of bab, corto, and crm at the locus containing TH in polytene chromosomes suggest that they might all cooperate in the direct regulation of this pigmentation enzyme, and that they might counteract the effect of temperature and Abd-B on TH expression. Their mutations enhance the repression of TH by Abd-B and high temperature, which explains why it has a particularly strong effect in A6 and A7, at 25 °C and 29 °C. We therefore propose the model presented in Figure 8 to explain some aspects of the pigmentation pattern plasticity in the posterior abdomen.

It does not exclude that temperature also modifies the expression of other genes. This is likely as the PcG/TrxG have hundreds of targets [38,39], and the thermosensitivity of the PcG/TrxG system is a general phenomenon observed with PRE from several different genes [38,44]. It is possible that other genes (developmental regulators or structural genes) are also modulated by temperature and contribute to the phenotypic plasticity of pigmentation. We observed, indeed, many colocalizations of Corto and BAB, and Corto and CRM.
suggesting that this particular network of chromatin regulators regulate many targets. We demonstrate that this network is involved in at least two different phenotypes, abdominal pigmentation in females and sex comb development in males, both showing high temperature sensitivity. Thus, we propose that the plasticity of Drosophila pigmentation is a visible side effect, at particularly sensitive loci, of a process affecting the whole genome through alteration of epigenetic mechanisms.

Interestingly, abdominal pigmentation and morphology of the sex comb along the proximo-distal axis of the first leg evolve very rapidly in the Drosophila genus [57–59]. Remarkably, we found that these two morphological traits are under the control of a common thermosensitive network including the transcription factor bab, chromatin regulators, and the chaperone Hsp90. This suggests that the thermosensitivity of this particular regulatory network might be linked to the high evolvability of several secondary sexual characters in the genus Drosophila. Our results corroborate other studies, which have shown that the plasticity of specific traits is correlated to their evolvability [60].

Materials and Methods

Origin and maintenance of fly stocks, crosses. Most of the fly stocks used in this study were provided by the Bloomington Drosophila Stock Center (http://flystocks.bio.indiana.edu). The following ones were kindly sent to us by various researchers: babΔ505 and UAS-bab1 (Jean-Louis Coutere), crm (Neel Randahl), Df(3R)RS1-98Db-P5 (Arytym Koppp), ebony-LacZ (Bernard Hovemann), and Pβ-gal (Ian Duncan). The Tab mutation is associated with the Pc3 inversion in the stock we used, but the pigment phenotype visible on the thorax has been shown to be caused by the ectopic expression of Abd-B [17]. Flies were grown on standard agar-corn medium. Standard balancer chromosomes were used. For the effect of temperature, crosses were kept at 25 °C and tubes transferred after 2 d to the desired temperature. Oregon-R was used as a wild-type stock to outcross mutant lines. All fly stocks are described in Flybase (http://flybase.bio.indiana.edu).

The interaction between bab, chromatin regulators, and chaperones was analyzed using sex comb phenotypes (except for the babΔ505 interaction) and female abdominal pigmentation in the progeny of crosses between females Oregon-R or babΔ505/+ and males carrying the mutation to be tested, or wild-type Oregon-R males. The interactions between crm (located on the X chromosome) and bab on sex comb was analyzed in the male progeny of crosses between crmΔ7FM7c females and males babΔ505/TM6b, and compared to the effect of these mutations alone when crossed to Oregon-R. The effect and interaction with corto and bab of the hspb83Δ/+ allele on male abdominal pigmentation was observed and analyzed in the male progeny of crosses made with females hspb83Δ/+TM6b and wild-type males or carrying the mutation to test.

Abdominal and thoracic cuticle preparation. Flies were fixed in 75% ethanol 3 d after hatching to allow proper pigmentation of the pharate adults according to progression of eye color, bristle and wing melanization, meconium appearance, and ability of the fly to walk properly. Pupae were dissected out of the pupal case for the babΔ505 case [64]. Standard balancer chromsomes were used. Flies were fixed in 37 °C for 3 h for e-LacZ and ddc-LacZ, or 2 h for TH-GAL4; UAS-LacZ genotypes. Thoraces or abdomen were dissected after staining in PBS. This allowed us to make sure that absence of staining was not caused by tissue disruption during dissection prior to staining. Tissues were then dehydrated in min in 75% ethanol, 5 min in 100% ethanol, and mounted in Euparal.

Polypeptide chromosomes. Immunostaining of polypeptide chromosomes was performed as described by Cavalli (http://www.igh.cnrs.fr/equip/cavalli.html) on larvae of the w1118 genotype. The rabbit anti-CRM [35], rat anti-BAB2 [30], and rabbit anti-Corto [65] antibodies were used respectively, at 1:50, 1:200, and 1:25 dilutions.

Statistical analysis of sex comb tooth number and pigmentation phenotypes. All statistical analyses were performed using the software SPSS 10.0 or SPSS 13 [66]. We scored sex comb tooth number in the ectopic sex comb on the dorsal midline (A6D), on both sides in the lateral region of A6 (A6L1, A6L2), and in the median region of A6 (A6M1, A6M2) (Figure S2). In A7 we scored the proportion of melanin on both sides along the dorso-ventral axis (A7DV, A7DV2) and the lateral region along the antero-posterior axis (A7L1, A7L2) (Figure S2). Individuals were scored for each genotype/temperature combination. Pigmentation scores varied between 0 (no melanin) and 4 (fully black) (Figure S2). Pigmentation scores between the left and right side were highly correlated within all regions (all ρ > 0.925, p < 0.001). They were averaged in each individual (A6L, A6M, A7L, A7DV) and analyzed through multivariate analysis of variance. A6L, A6M, A6D, A7L, and A7DV were used as dependent variables; temperature and genotypes at Abd-B (one, two, or three doses), bab (one or two doses), corto (wild-type or cortoΔ+), Hsp83 (wild-type or Hsp83Δ+), and CRM (wild-type or CRMΔ+) as fixed factors. We included all main effects as well as possible interaction terms in the model (Table S1).

The model includes genes interacting with bab (encoding putative cofactors). Thus, in this model, although the effect of bab and the interaction between bab and temperature are highly significant (Table S1), they are also allocated to the interactions between bab and other genes, and between bab, other genes, and temperature. In order to test more generally the effect of bab and its interaction with temperature before dissecting the network (see Results), we also performed a multivariate analysis using the same dependant variables: temperature and genotype at bab as fixed factors in a reduced dataset with only wild-type and babΔ505/+ females (Table S2).

Supporting Information

Text S1. Consensus Model of Pigment Synthesis Pathway

Found at doi:10.1371/journal.pgen.0030030.sd.001 (63 KB DOC).

Figure S1. Abd-B and bab Interact with Temperature for the Regulation of Melanin Production

(A–J) Effect of modulation of Abd-B copy number on the plasticity of the abdominal pigmentation pattern in females: one dose (Df(3R)RS1–98), two doses (wild-type), and three doses (Dp-P5). (J–L) Effect of the reduction of bab expression level on the plasticity of the abdominal pigmentation pattern. Found at doi:10.1371/journal.pgen.0030030.sg001 (7.7 MB TIFF).

Figure S2. Drawing Illustrating How We Scored the Pigmentation Phenotypes

The pattern of melanin is differently affected by genetic factors and temperature in A6 and A7 and along the dorso-ventral axis. Thus, we scored the proportion of melanin in A6 along the antero-posterior axis at the dorsal midline (A6M), on both sides in the lateral region of A6 (A6L1, A6L2), and in the median region of A6 (A6M1, A6M2). In A7, we scored the proportion of melanin on both sides along the antero-posterior axis in the lateral region (A7L1, A7L2) and along the dorso-ventral axis (A7DV1, A7DV2). Pigmentation scores varied from 0 (no melanin) to 4 (fully black).

Found at doi:10.1371/journal.pgen.0030030.sg002 (363 KB TIFF).

Figure S3. Phenotypes of Pigmentation Mutants Grown at 20 °C, 25 °C, and 29 °C

(A–L) Female hemitergites of abdominal segments number 4 to 7. (M–
Figure S4. Thoracic Pigmentation Phenotypes and Expression of Pigmentation Enzymes

Thoracic phenotype of a wild-type (Oregon-R) fly (A), an antioxidant (B), and a yellow mutant (C-D). (A) Thoracic expression of yellow hydroxylase visualized with TH-Gal4; UAS-LacZ. (E) Thoracic expression of ddc-LacZ. (F) Thoracic expression of ebony-LacZ. All flies were grown at 25 °C. Note that all three enzymes are upregulated in the pattern of the thorax visible in e1 heterozygous and double-mutants.

Figure S5. bab Represses ddc

shows abdominal phenotype of the ectopic expression of bab1 in the dorsal domain using the driver Panier-Gal4 and UAS-bab1 in an ebony (A) and yellow (B) background. Flies were grown at 20 °C because the gain-of-function experiment was lethal at higher temperature. They are thus very pigmented. Pigments disappear in the dorsal domains in both experiments (arrows). (C) Expression of the ddc-LacZ at 20 °C in female abdomen. (D) Expression of ddc-LacZ at 20 °C in a female overexpressing bab1 in the dorsal domain. Note the downregulation of LacZ in the dorsal domain.

Figure S6. Chromatin Regulators, bab, and the Chaperone Hsp90 Interact with Temperature in the Regulation of Melanin Production

Abdominal pigmentation phenotypes in females carrying combinations of mutation in the transcription factor bric-a-brac (bab), the chromatin regulators corto or cramped (crm), or Hsp83, encoding the chaperone Hsp90, grown at 20 °C, 25 °C, and 29 °C. See text for details.

Figure S7. Interactions with Other corio Alleles

Abdominal pigmentation phenotypes in females carrying combinations of mutation in the transcription factor bric-a-brac (bab), the chromatin regulator corto or Hsp83, encoding the chaperone Hsp90, grown at 20 °C, 25 °C, and 29 °C. See text for details.

Figure S8. Effect of the crm7 Allele on Melanin Production in Female Abdomen

Abdominal pigmentation phenotypes of a crm7 homozygote female, a crm7/FM7C sibling heterozygote with the balancer from the stock, and a heterozygote crm7 female obtained in a cross with the wild-type line Oregon-R. Note the strong reduction of melanin production in abdominal segments A6 and A7 in (A). Found at doi:10.1371/journal.pgen.0030030.sg007 (3.1 MB TIF).

Figure S9. Abdominal Phenotypes of Males Heterozygote for the hsp83280 Allele of the Gene Encoding the Chaperone Hsp90 in an otherwise Wild-Type (A–C), corto2840/28+ (D–F), corto2840/28+ (G–I), or bab2840/28+ (7–9) Background

All genotypes were grown at 20, 25, and 29 °C. Small patches where melanin production is abnormal are indicated in (B and C, arrows). Males heterozygous for corto2840/28+ and corto2840/28+ and bab2840/28+ have a normal pigmentation in A5 and A6 at all three temperatures (unpublished data).

Found at doi:10.1371/journal.pgen.0030030.sg009 (5.4 MB TIF).

Table S1. Multivariate Analysis of the Effect of Temperature and Particular Regulatory Genes on Melanin Production

We analyzed the lateral region of A6 (A6L), the median region of A6 (A6M), the dorsal midline of A6 (A6D), the lateral region of A7 (A7L), and along the dorso-ventral axis in A7 (ATDV).

Found at doi:10.1371/journal.pgen.0030030.st001 (22 KB DOC).

Table S2. Multivariate Analysis of the Effect of Temperature and the Gene bab on Melanin Production

We analyzed the lateral region of A6 (A6L), the median region of A6 (A6M), the dorsal midline of A6 (A6D), the lateral region of A7 (A7L), and along the dorso-ventral axis in A7 (ATDV).

Mean and standard errors of sex comb teeth number and sample size of individuals scored. Unlike bab1+/+, the other mutations do not induce the formation of ectopic sex comb on the second tarsal segment, so we analyzed how they modify the bab1+/+ sex comb phenotype.

Found at doi:10.1371/journal.pgen.0030030.st002 (22 KB DOC).

Table S3. Effect of Mutations of bab and Chromatin Regulators in Males on Sex Comb Teeth Number on the Second Tarsal Segment of the First Leg

Effect of Mutations of bab and Chromatin Regulators in Males on Sex Comb Teeth Number on the Second Tarsal Segment of the First Leg

Found at doi:10.1371/journal.pgen.0030030.st003 (48 KB DOC).

Table S4. Two-Way Analysis of Variance of the Effect of Genotype and Temperature on Sex Comb Teeth Number on the Second Tarsal Segment of the First Leg

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