Genetica (2009) 135:403–413
DOI 10.1007/s10709-008-9286-2

Phenotypic plasticity of abdomen pigmentation in two geographic populations of *Drosophila melanogaster*: male–female comparison and sexual dimorphism

P. Gibert · B. Moreteau · J. R. David

Received: 7 November 2007 / Accepted: 11 June 2008 / Published online: 22 June 2008

Abstract In *Drosophila melanogaster* male, the last abdominal tergites (A5–A6) are completely dark due to a strong internal constraint while, in female, all abdominal tergites (A2–A7) are phenotypically variable and highly plastic. Male A2–A4 are quite similar to those of female, but their plasticity was never investigated. In this paper, we compared the phenotypic plasticity of A2–A4 in both sexes in order to know if the major dimorphism (SD) expressed in male A5–A6 also extended toward the more anterior segments. We also compared two geographic populations living under very different climates in order to know if adaptive differences, previously observed in females also existed in males. With an isofemale line design, pigmentation variation according to growth temperature was investigated in the two populations from France and India. Male and female data were compared and sexual dimorphism (SD) analyzed in various ways. Reaction norms were quite similar in both sexes for A2 and A3, but clearly different for A4. Considering the total pigmentation (A2 + A3 + A4) males were darker than females at low temperatures and either identical to them (France) or lighter (India) above 25°C. SD (male–female difference) was genetically variable among lines and significantly different among segments. Reaction norms of SD exhibited an overall decrease with temperature and also a significant difference among populations, suggesting a local adaptation of SD to thermal conditions. The three plastic segments in male (A2–A4) seem to react adaptively to the thermal environment more efficiently than the same segments in female, in agreement with the thermal budget hypothesis. To our knowledge, it is the first time that a SD trait exhibits an adaptive difference between geographic populations.

Keywords Climatic adaptation · Modular trait · Isofemale lines · Reaction norms · Growth temperature · Tergites melanisation

P. Gibert (✉)
Laboratoire de Biométrie et Biologie Evolutive, Université de Lyon, Université Lyon 1, CNRS, UMR 5558, 43 boulevard du 11 novembre 1918, Villeurbanne 69622, France
e-mail: gibert@biomserv.univ-lyon1.fr

B. Moreteau · J. R. David
Laboratoire Evolution, Génome et Spéciation, CNRS, Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France

B. Moreteau · J. R. David
Université Paris-Sud 11, 91405 Orsay Cedex, France

J. R. David
Department Systematique et Evolution, Museum National d'Histoire Naturelle, UMR 5202 (OSEB), 45 rue Buffon, 75005 Paris, France

Introduction

Temperature is a most important component of the abiotic environment for understanding the distribution and abundance of ectotherm species (Andrewartha and Birch 1954; Precht et al. 1973; Cossins and Bowler 1987; Hoffman and Parsons 1991; Leather et al. 1993). Numerous life history traits, such as rate of development, viability, progeny production, longevity, have been measured in many species in relation with ambient temperature. All these results contribute to our understanding of geographic distributions. Even in most investigated species, such as Drosophila, our knowledge is, however, far from complete (David et al. 1983).

At an intraspecific level, numerous Drosophila species exhibit latitudinal clines, i.e. regular genetic variations,

which are assumed to reflect adaptive responses to local climatic conditions. In *D. melanogaster*, latitudinal clines have been observed for various morphometrical traits such as body size, brisle numbers, ovariole number or body pigmentation (see David and Capy 1988; Gibert et al. 2004a, for reviews). In the case of pigmentation, latitudinal clines have been demonstrated for a dark trident pattern on the mesonotum (David et al. 1985, Capy et al. 1988; Munjal et al. 1997). A genetic difference, i.e. lighter flies in a warmer environment, is also evidenced for abdomen pigmentation (Gibert et al. 1998a). Body pigmentation is also very plastic in relation to growth temperature (David et al. 1990; Gibert et al. 1996) and both clinal and plastic variations produce darker flies in a cold environment. Such a phenomenon, observed also in other insects, is generally explained by the thermal budget hypothesis: a dark color is beneficial in the cold for collecting the energy of solar radiations and increasing body temperature (Watt 1969; Gibson and Falls 1979; David et al. 1985, 1990; Kingsolver and Wiernasz 1991; Gibert et al. 1996; Majerus 1998; True 2003).

In *D. melanogaster,* the pigmentation variability is also expressed among abdomen segments. Each tergite reacts to developmental temperature with a specific response curve (the reaction norm) (Gibert et al. 1998b, 2000, 2004b). All studies on abdomen pigmentation have been done, up to now, on females only. The reason is that all visible segments from A2 to A7 exhibit a similar pattern on each tergite, with a black melanin area on the posterior part. Males, on the other hand, exhibit as a secondary sexual character, a black pigmentation on the last two abdomen segments, A5 and A6, hence the name of *melanogaster*. This male specific pigmentation is not plastic and its genetic basis corresponds to the inhibition in male of the expression of a transcription factor *bric a brac* (*bab*) (Kopp et al. 2000, 2003). Males tergites A2 to A4 exhibit, however, a pigmentation pattern similar to that seen in female, and the extension of the black area is also very plastic (unpublished observations), although it was never precisely investigated.

We present here a comparative analysis of genetic variability and phenotypic plasticity in segments A2–A4 of both sexes of *D. melanogaster,* using an isofemale line design (David et al. 2005). We also compare two distant geographic populations, a French one (Bordeaux) living under a mild humid temperate climate and an Indian one (Rohtak) adapted to a tropical climate with a very hot and arid summer. Previous investigations already evidenced an overall lighter pigmentation in Rohtak females (Gibert et al. 1998a, 2004b) as well as a better tolerance of spermatogenesis to heat stress (Rohmer et al. 2004). As a control, we also measured the thoracic (trident) pigmentation, which is known to be very similar in both sexes but different among populations (David et al. 1985; Capy et al. 1988; Munjal et al. 1997).

Besides the need to fill a gap in our knowledge concerning abdomen phenotypic plasticity in *D. melanogaster* male, we tried to answer more specific questions. (1) What is the shape of the reaction norms of tergite pigmentation in male A2–A4? (2) Is there a difference in plasticity between sexes and is the sexual dimorphism itself a plastic trait? (3) Can we find possible adaptive differences in males between populations living under very different climates?

Our data show that a significant difference in sex dimorphism between the two geographic populations may be explained by assuming that the male anterior segments (A2–A4) are more strongly selected than those of the female.

Material and methods

Populations investigated and experimental procedure

A temperate population from Bordeaux (southern France) was compared to a tropical population from Rohtak, near Delhi (India). While Bordeaux is typical of a cool temperate locality with mild summer and cold winter, Rohtak is typical of a subtropical arid climate with mild winter but very hot summer. A set of 20 lines was randomly taken from each locality and the phenotypic plasticity of female abdomen pigmentation was investigated by two different observers (Gibert et al. 1998a, 2004b). Each observer analyzed 10 lines from France and 10 lines from India. An eventual comparison did not evidence any significant observer effect. One observer also analyzed the male pigmentation in the two sets of 10 lines and the results are presented in this paper. For comparing the two sexes, only the lines for which males and females data are available, are considered, that is 10 lines from each locality. After adult emergence, 10 pairs of the first laboratory generation were used as parents for each line. They oviposited for a few hours at 21°C, in a vial containing a high nutrient, killed yeast medium (David and Clavel 1965). Population density was not precisely controlled, but was generally less than 150 individuals per vial. Moreover we know that, with such a rearing medium, larval density fluctuations have no significant effect on adult phenotype (Karan et al. 1999). Immediately after parent's removal, vials were transferred to a constant temperature incubator regulated at ±0.1°C. The procedure was repeated several times over successive days and the progeny of each line was grown at 7 temperatures, namely 12, 14, 17, 21, 25, 28 and 31°C. For the heat tolerant population of Rohtak, we could also obtain a sufficient number of flies at 32.5°C, and this temperature was added to the set of 7 temperatures common for the two

populations. After emergence, adult flies (second laboratory generation) were transferred to fresh vials and stored at a medium temperature (21 or 17°C) until phenotype pigmentation could be measured.

Traits analysed

For the abdomen pigmentation, we estimated visually the extension of the dark stripe of black pigment found at the posterior margin of each tergite. We used 11 phenotypic classes, ranging from 0 (no visible dark pigment) up to 10 (tergite completely black). For a precise illustration, see David et al. (1990). In both sexes, segment A1 is small and its pigmentation, if any, is difficult to score, so it was not considered in our study. For the female, we scored six successive segments, from A2 to A7. In males, only 6 segments have a well developed tergite. Moreover, the last two segments (A5 and A6) are completely black. In fact this is not an absolute rule, and a precise observation showed indeed that segment A5 was not always 100% black. So we scored as a routine segments A2 to A6, segments A2, A3 and A4 being the most phenotypically variable, in a way quite similar to what is found in females.

D. melanogaster is also known to exhibit a darker pattern with a trident shape on the mesonotum. We estimated the pigmentation of this thoracic trident, using 4 phenotypic classes only, ranging from 0 (no visible trident) to 3 (very dark trident). See David et al. (1985) for a more detailed description. For comparison with the pigmentation score of the abdomen segments, the thoracic score was then multiplied by 3.33, so that it also ranged from 0 to 10.

Data analysis

The phenotypic plasticity of pigmentation was analysed by considering for each segment the response curve, or reaction norm, of dark pigmentation in relation to developmental temperature. Female reaction norms of A2–A4 are significantly different and can be adjusted to quadratic polynomials (see Gibert et al. 2000). We calculated for each line the coordinates of the minimum providing three parameters: the minimum value (MP) which is a property of the trait and two characters related to plasticity, i.e. the temperature of minimum value (TMP) and a curvature parameter called g_2 (see David et al. 1997, 2006a for technical precisions).

For each sex and temperature, the variability among lines can be compared to the within line variability, permitting the calculation of an intraclass correlation which is an estimate of the genetic variability (Hoffmann and Parsons 1988; Capy et al. 1994; David et al. 2005). Genetic correlations were estimated, as in previous works, from family means (Via 1984; Gibert et al. 1998b; Kopp et al. 2003).

Since phenotypic plasticity of males was never investigated, we focused on that sex, compared the results to those of females and analyzed for each segment the sexual dimorphism (SD). There are two major ways to describe SD, either among species or among full-sib lines: either the female-male difference in the mean values, or a ratio such as the female/male ratio (see David et al. 2003, 2006b). For a metrical trait such as thorax length, a ratio appears a more convenient estimate (Reeve and Fairbairn 1999; David et al. 2003, 2006b; Huey et al. 2006) since it is not sensitive to scaling effects. For pigmentation, the utilization of a ratio was not always convenient because, at extreme temperatures, the pigmentation might be sometimes completely black or completely light (scores of 10 or 0). It turned out that the difference between sexes was generally a better descriptor of the dimorphism, especially because male and female pigmentation was expressed on the same scale. For convenience, we choose the male minus female (M–F) difference. These sex differences exhibited specific reaction norms according to developmental temperature, which were also analysed by polynomial adjustment.

SD being measured on two different kinds of flies, it seems at first sight impossible to obtain an estimate of its variability at the individual level, and hence to calculate an heritability. In a previous work, this difficulty was overcome by dividing, within each line, each female value by the mean of their brothers (David et al. 2003). The availability of a within-line and a between-line variance allowed the calculation of an intraclass correlation. In the present work, we used a similar technique. For instance, the M–F difference within-line was estimated by subtracting, from each male value, the average pigmentation score of their sisters. Within and between line variances could thus be estimated, permitting the calculation of an intraclass correlation for SD.

Results

Reaction norms of pigmentation in both sexes

The reaction norms of female pigmentation of thoracic trident and of abdomen tergites A2 to A7 were analysed in a previous paper (Gibert et al. 2000). We present here the reaction norm observed in males, for the trident and segments A2 to A6 (Fig. 1) and they are compared to female norms. For the sake of simplicity, data of a single population, Bordeaux, are presented but the results of the Rohtak population exhibit an overall similarity. For the two anterior abdomen segments (A2 and A3) and for the trident, the results in both sexes are quite similar. A greater difference is found for segment 4, males being darker than females at all temperatures. As expected, the sex difference

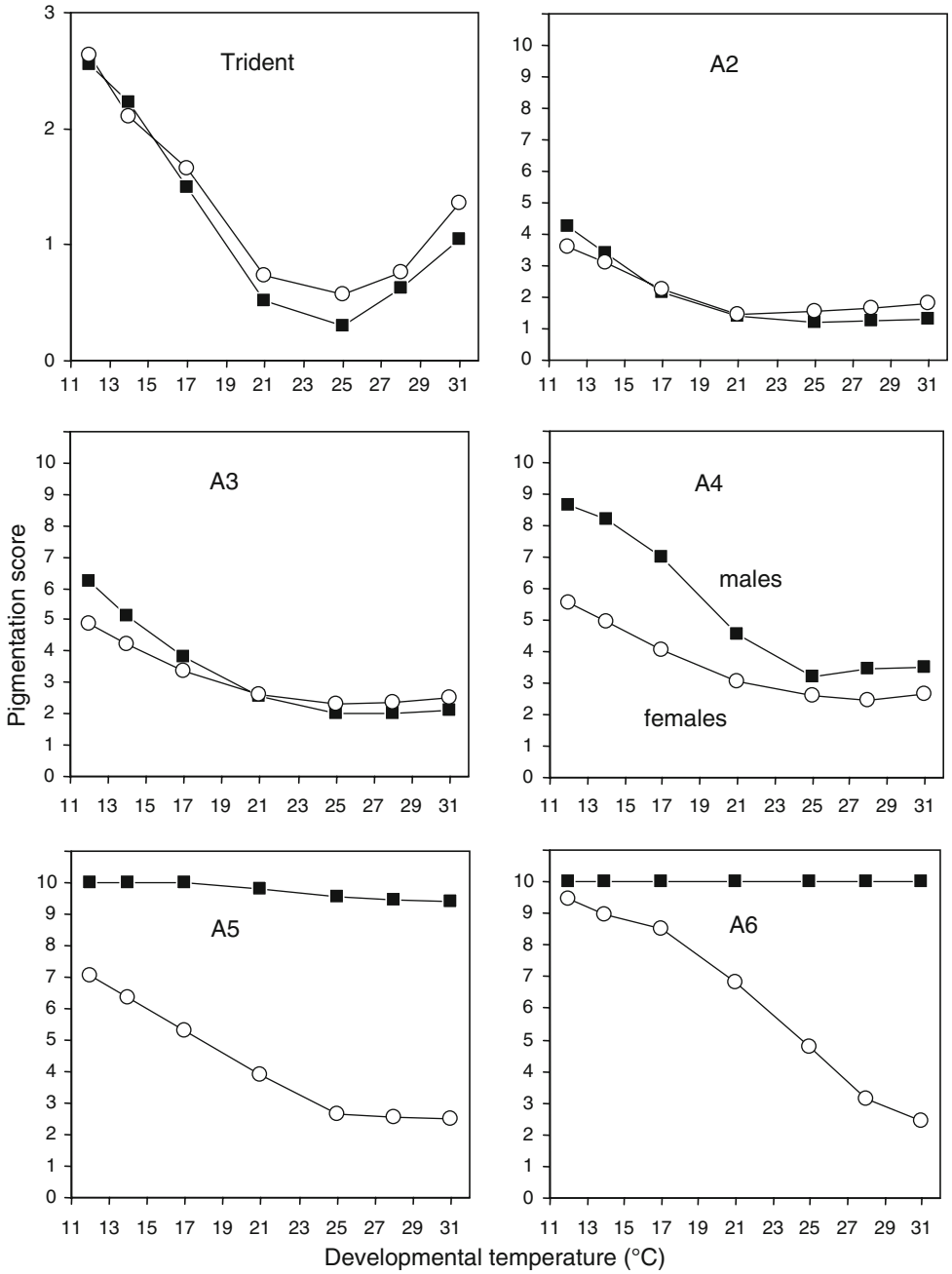

Fig. 1 Pigmentation reaction norms of thoracic trident and abdomen segments (A2–A6) according to developmental temperature in males and females of the Bordeaux population. Each point corresponds to the mean of 10 isofemale lines. Males are in black squares and females in white circles

is much greater for segments A5 and A6. The last tergites in males are black, and do not respond to growth temperature. This corresponds to the well known sexual dimorphism of *D. melanogaster*. In fact, the lack of phenotypic plasticity is mainly valid for segment A6, while segment A5 shows a very slight but significant decrease of the black area with increasing developmental temperature. For the females, on the other hand, the corresponding segments are always lighter and exhibit a large amount of plasticity. The same conclusion is valid for segment A7, which has no counterpart in male. If we want to analyse the plasticity of pigmentation SD, we should consider only the segments, which exhibit similar levels of plasticity in both sexes, i.e. the pigmentation of the thorax and of abdomen segments A2, A3, A4. ANOVA (not shown) revealed highly significant effects of segment, sex, temperature and population.

A means to synthesize the comparison of the two populations is to sum up the abdomen data A2, A3 and A4 into a single value. The reaction norms of the sum for the two populations are presented in Fig. 2, with two main conclusions. Firstly, the males from Bordeaux are, on average, darker than those of Rohtak. Their pigmentation score decreases, according to growth temperature, from 19 to 6,

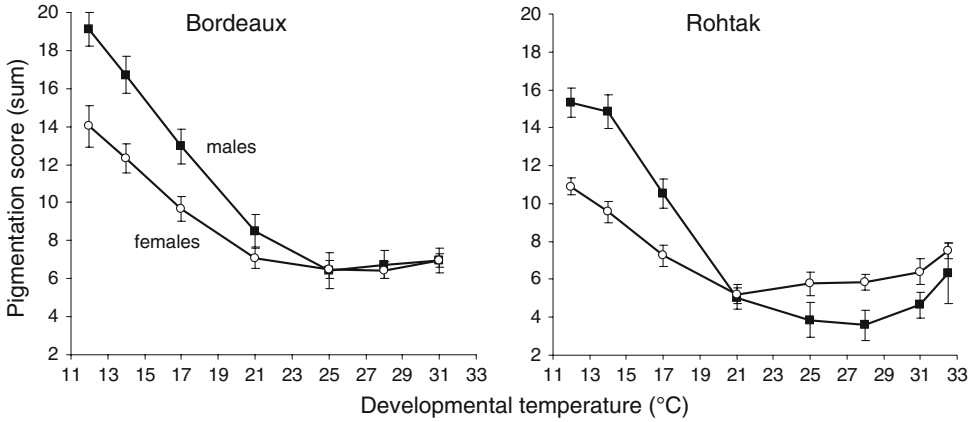

Fig. 2 Reaction norms of pigmentation score according to developmental temperature in males and females for the sum of the three anterior abdominal segments (A2 to A4) in the two populations France (**a**) and India (**b**). Males are in black squares and females in white circles. Vertical bars correspond to confidence intervals

while the corresponding values in Rohtak are 15 to 4. A similar difference (lighter pigmentation in Rohtak) is observed in the females, in agreement with previous observations (Gibert et al. 1998a). Secondly, the reaction norms are quite different. In Bordeaux, males are darker than females at low temperatures and become identical to them at 25°C and above. Males are also darker than females at low temperatures in Rohtak, but a reverse difference (darker females) is observed at high temperature. This result arises, at least partly, from a low level of phenotypic plasticity in the Rohtak females. In the statistical analysis, this difference results in a highly significant sex*population interaction.

Data of each isofemale line were adjusted to a quadratic polynomial: $y = MP + g_2(t\text{-}TMP)^2$, which was used to calculate characteristic values, that is the minimum pigmentation (MP) which defines the trait, and two other values, the temperature of minimum pigmentation (TMP) and a curvature parameter g_2 which both are relevant to plasticity (see David et al. 2006a for details). A high value of g_2 means that the curvature of the reaction norm is more pronounced (a highest phenotypic plasticity) while a low value of g_2 corresponds to a more flattened reaction norm. A comparison between males and females revealed only four significant differences. Three concerned the curvature parameter g_2, which was higher in males than in females for segments A2, A3 and A4 in the Rohtak population. A similar, although non-significant effect was observed in Bordeaux. We investigated more precisely the variation of the curvature (g_2) according to sex and segments position. For a clearer presentation (Fig. 3) data of Bordeaux and Rohtak were pooled. The curvature parameter, g_2 is much more pronounced for the thoracic trident than for the three other abdomen segments in both populations with no difference between sexes (see also Fig. 1). For the three abdomen segments, curvatures are much less than for the thorax. In the females, we notice a slight, non-significant increase between A2 and A4. Curvatures are significantly greater in males for all segments, with a maximum difference for A4.

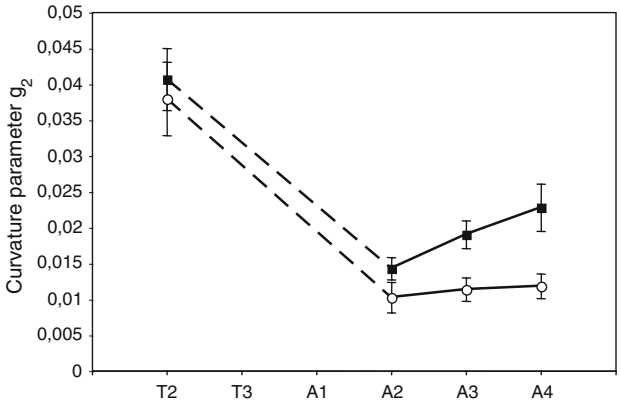

Fig. 3 Variation of the curvature parameter (g_2) of the reaction norms in body segments of female and male. Data of the two populations were pooled. Vertical bars indicate the confidence intervals. T2, T3: segments 2 and 3 of thorax; A1 to A4: abdomen segments 1–4. Pigmentation was observed only on thorax 2 (trident) and abdomen segments 2–4

Another significant difference between sexes concerns the minimum pigmentation of segment A3 in Rohtak which is lighter in males. Finally we compared the two other characteristic values along the antero-posterior axis, and found major differences. The minimum pigmentation (MP) increased toward the posterior end in both sexes and the Rohtak population exhibited significantly lesser values than Bordeaux. The temperature of minimum value (TMP) also increases backwards, from 25.0 ± 0.3 (trident) to 29.3 ± 0.8 (A4) ($n = 4$ in each case). There was however no significant difference between the two populations nor between sexes.

Genetic variability of pigmentation: intraclass correlations

The amount of genetic divergence between lines was estimated for each segment, temperature, population and sex by the coefficient of intraclass correlation (Hoffman

and Parsons 1988; David et al. 2005) (Table 1). A four way ANOVA (not shown) applied to these data revealed significant differences between trait and temperature, while the direct effects of population and sex were not significant. For the sake of simplicity, we give only the average values for both populations in Table 1. Significant interactions were however observed, namely sex-by-trait, sex-by-population, sex-by-temperature and population-by-temperature. The trait effect is mainly explained by a low heritability of the thoracic trident, especially in males. The temperature effect does not correspond to a regular trend, the lowest value (0.17 ± 0.03) being observed at 17°C and the highest at 25°C (0.31 ± 0.04) (n = 16 in each case). Finally, the overall mean values in both sexes are very similar: 0.22 ± 0.01 in females and 0.20 ± 0.02 in males (n = 28 in each case).

Table 1 Coefficients of intraclass correlations observed at different temperature for the pigmentation of different segments

Temp. (°C)	Trid.		A2		A3		A4		Sum	
	m	f	m	f	m	f	m	f	m	f
12	0.15	0.18	0.19	0.35	0.10	0.29	0.10	0.28	0.11	0.36
14	0.11	0.21	0.25	0.37	0.17	0.22	0.23	0.23	0.23	0.38
17	0.13	0.22	0.24	0.26	0.19	0.10	0.13	0.13	0.19	0.19
21	0.10	0.21	0.28	0.14	0.28	0.28	0.16	0.23	0.26	0.21
25	0.12	0.15	0.48	0.31	0.28	0.28	0.38	0.24	0.50	0.33
28	0.03	0.26	0.31	0.15	0.08	0.08	0.32	0.14	0.37	0.16
31	0.22	0.22	0.15	0.22	0.24	0.24	0.25	0.26	0.27	0.28
Mean	0.12	0.21	0.26	0.26	0.28	0.21	0.23	0.22	0.29	0.27
SE	0.02	0.01	0.04	0.03	0.05	0.03	0.04	0.02	0.04	0.03

Values obtained for the two populations were not significantly different ($F_{(1,18)} = 1.69$ ns) and averaged. m: male, f: female

Trid: thoracic trident; A2, A3, A4: abdominal segments 2–4, Sum: A2 + A3 + A4

Analysis of sexual dimorphism

Genetic correlations between sexes

We calculated genetic correlations between males and females for the different characters, temperatures and populations (Table 2). Among 75 coefficients, most of them (72) were positive and 20 were significant after a Bonferroni correction. Results were submitted to ANOVA after a z transformation, and the conclusion was a highly significant trait effect ($F_{(3,18)} = 8.19$***). The higher correlation was found for the thoracic trident (r = 0.75 ± 0.06). No significant differences were found between temperatures and populations but a highly significant population*temperature interactions ($F_{(6,18)} = 5.07$**) was observed. Correlations at high temperatures tended to be lower in Bordeaux than in Rohtak.

Average reaction norms

Average reaction norms of pigmentation SD (male–female) are shown Fig. 4 for segments A2, A3, A4 and

Table 2 Genetic correlation between sexes for each population, temperature and trait

	T°C	Tri.	A2	A3	A4	Sum	mean ± SE
Bdx	12	0.78 NS	0.57 NS	0.59 NS	0.68 NS	**0.82***	0.68 ± 0.05
	14	0.25 NS	0.50 NS	0.44 NS	0.13 NS	0.12 NS	0.28 ± 0.08
	17	**0.83***	**0.83***	0.78 NS	0.70 NS	**0.80***	0.79 ± 0.02
	21	**0.86***	0.39 NS	0.54 NS	0.50 NS	0.55 NS	0.57 ± 0.08
	25	**0.81***	0.24 NS	0.39 NS	0.28 NS	0.41 NS	0.43 ± 0.10
	28	**0.93*****	0.22 NS	0.39 NS	0.67 NS	0.46 NS	0.53 ± 0.12
	31	**0.82***	0.44 NS	−0.32 NS	0.22 NS	−0.06 NS	0.22 ± 0.20
		0.75 ± 0.09	0.46 ± 0.08	0.40 ± 0.13	0.45 ± 0.09	0.44 ± 0.12	0.50 ± 0.06
Roh	12	0.31 NS	−0.06 NS	0.14 NS	0.41 NS	0.16 NS	0.19 ± 0.08
	14	0.66 NS	0.70 NS	0.29 NS	0.60 NS	0.59 NS	0.57 ± 0.07
	17	**0.89***	0.57 NS	0.31 NS	0.12 NS	0.46 NS	0.47 ± 0.13
	21	**0.84***	0.64 NS	0.39 NS	0.57 NS	0.42 NS	0.57 ± 0.08
	25	0.74 NS	**0.89***	**0.89***	0.74 NS	**0.88***	0.83 ± 0.04
	28	**0.85***	0.71 NS	0.40 NS	0.36 NS	0.54 NS	0.57 ± 0.09
	31	**0.86***	**0.83***	**0.81***	0.69 NS	**0.81***	0.80 ± 0.03
	32.5	**0.93***	**0.80***	0.63 NS	0.61 NS	0.72 NS	0.74 ± 0.06
		0.76 ± 0.08	0.64 ± 0.11	0.48 ± 0.10	0.51 ± 0.08	0.57 ± 0.09	0.59 ± 0.05

Each coefficient is based on 10 lines. Significant coefficients are marked in bold. *P*-values are corrected by a sequential Bonferroni-type procedure

*** $P < 0.001$, * $P < 0.05$, NS: non-significant

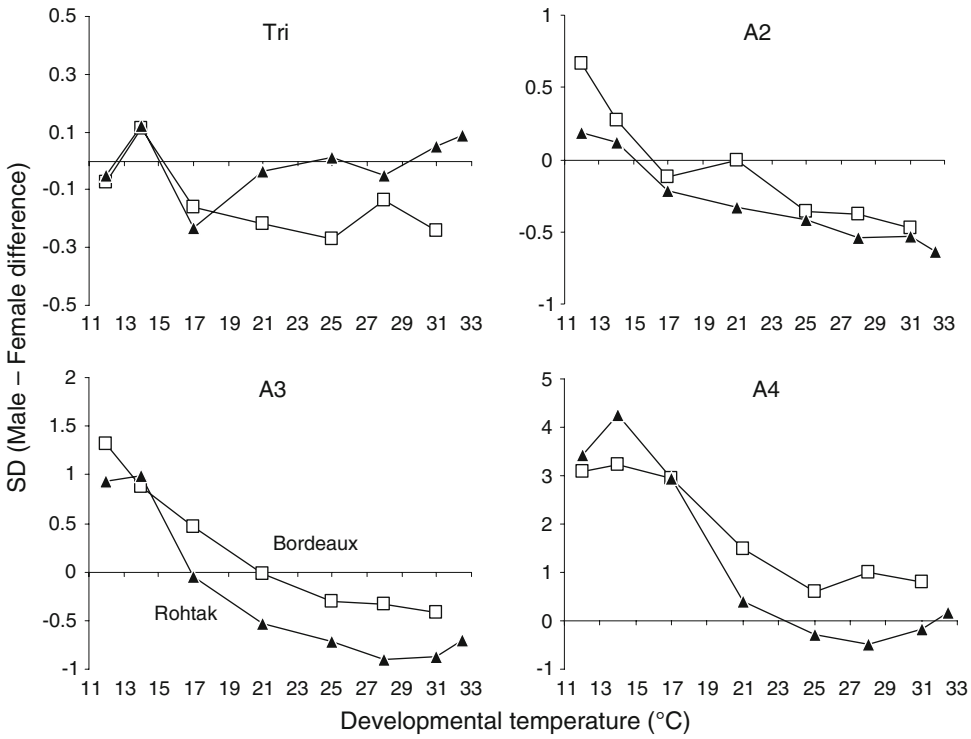

Fig. 4 Reaction norms of sexual dimorphism estimated by the difference between male and female pigmentation. Bordeaux (France) is in white squares and Rohtak (India) in black triangles (notice the different scales used for SD)

for the trident, and in Fig. 5a for the sum. For each character, results were analysed by ANOVA (not shown). For the thoracic trident, the difference between sexes is very small in agreement with previous data (Munjal et al. 1997) and not influenced by temperature. For the three abdomen segments a decrease of SD with increasing temperature is observed. At low temperatures, males are darker than females (M–F difference is positive). In the case of A2 and A3, negative values are observed at warmer temperatures above 20°C: males are lighter than females. For A4, a similar trend is observed but significant negative values are not observed. For the three abdomen segments, the dimorphism is, on average, greater in Bordeaux than in Rohtak, but the difference is significant only for A3 and A4. To our knowledge, this is the first time that a dimorphism changes its sign over a thermal range, and this raises an interpretation problem. If the difference is zero, this means there is no dimorphism. If however, the difference shows a big, positive or negative value, this means that SD is very pronounced. In other words, the magnitude of SD should be expressed as the absolute value of the difference. Doing this, however, raised another problem. When the mean SD is equal to zero, taking the average absolute value will produce a significantly positive mean. Therefore, the absolute value transformation is valid only when SD is significantly

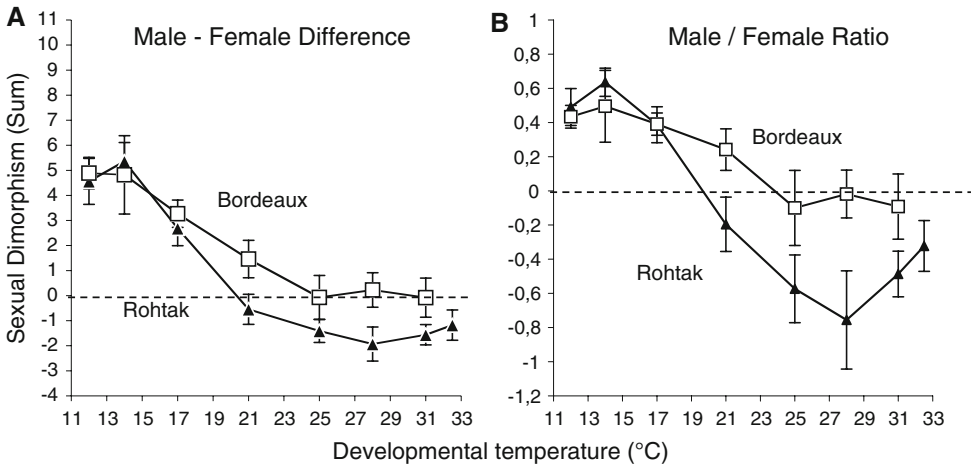

Fig. 5 Reaction norms of sexual dimorphism using different estimators for the sum of the first three abdomen segments (A2 + A3 + A4). (**a**) average reaction norm for the difference between male and female in the two populations, (**b**) reaction norms of the M/F ratio, after a $\log_2$ transformation. Vertical bars correspond to the confidence intervals. Bordeaux (France) is in white square and Rohtak (India) in black triangle

different from zero, and in that case, only the mean value, not those of each line, are to be considered. For the sum A2–A4 (Fig. 5a), we see that the dimorphism is maximum at low temperatures, with similar values in the two populations, males being darker than females. The dimorphism decreases progressively at higher developmental temperature, becoming nil in Bordeaux, while still significant (in an opposite direction) in Rohtak.

We also present the average values of the male/female ratio after a log transformation (Fig. 5b). The reaction norms of the male/female ratios are similar to those of Fig. 5a. The biological significance remains the same: males are darker than females at low temperatures with no difference between populations; a decrease of SD with increasing temperature is accompanied by the appearance of a divergence between populations: in Bordeaux, males and females become identical, while males in Rohtak become lighter than females.

Genetic variability of sexual dimorphism

For each segment, temperature and population, we calculated the intraclass correlation of SD (M–F difference) (Table 3). ANOVA (not shown) revealed significant segment and temperature effects, and also a population*temperature interaction. The temperature effect is not regular, since very low values were observed at 17 and 31°C. The trait value is mainly explained by a lower value for the trident. The population*temperature interaction does not correspond to a regular trend. The overall intraclass coefficient is 0.17 ± 0.02, significantly greater than zero but less than the value found for the traits themselves.

Discussion

Body pigmentation variability in Drosophila has been investigated in many species and there is a general consensus to recognize that it is a fast evolving trait which is not strongly influenced by phylogenetic constraints (Gibert et al. 1996; Hollocher et al. 2000; Kopp et al. 2000; Moreteau et al. 2003; Wittkop et al. 2003; Klaczko 2006). An extreme case concerns the genus *Leucophenga* in which female and male pigmentations are sometimes so different that the two sexes were sometimes identified as different species (Bächli 1971).

In *D. melanogaster*, the basic pattern for each abdomen tergite is a yellow pigmentation with a dark stripe of eumelanin at the posterior margin. The extension of the black pigmentation in females is extremely variable, being influenced by temperature, segment identity and genotype, and is quite easy to quantify (David et al. 1990; Gibert et al. 2000). Up to now pigmentation variability was not investigated in male, the main reason being that the two posterior tergites, A5–A6, are almost completely black, due to a constraining sex dimorphism. In female, a transcription factor encoded by the *bric a brac (bab)* locus is expressed in all segments. Temperature acts on melanin production by modulating a chromatin regulator network (Gibert et al. 2007), interacting genetically with the chaperone Hsp90 and the transcription factor *bab*. In males, *bab* expression is repressed in A5–A6, due to the joint expression of *Abdominal-B* and *double-sex* (Kopp et al. 2000; Wittkop et al. 2003). Male anterior segments A2–A4 escape the constraining dimorphism expressed in the last two segments exhibit a plastic variability, and can be analysed by recording the extension of the black area. On average, segments A4 is

Table 3 Genetic variability of SD (male–female) among isofemale lines

		12°C	14°C	17°C	21°C	25°C	28°C	31°C	32.5°C	
Trid	Bdx	0.03	0.19	0.01	0.13	0.16	0.07	0.05		0.09 ± 0.03
	Roh	0.27	0.17	0	−0.02	0.03	0.12	0.01	−0.04	0.07 ± 0.04
A2	Bdx	0.18	0.26	0.11	0.31	0.54	0.29	0.14		0.26 ± 0.05
	Roh	0.42	0.16	0.14	0.16	0.25	0.28	0.13	0.02	0.20 ± 0.04
A3	Bdx	0.09	0.15	−0.02	0.19	0.37	0.11	0.31		0.17 ± 0.05
	Roh	0.14	0.17	0.25	0.32	0.15	0.52	0.12	0.28	0.24 ± 0.05
A4	Bdx	0.09	0.24	0.08	0.13	0.27	0.06	0.15		0.15 ± 0.03
	Roh	0.06	0.16	0.1	0.07	0.29	0.44	0.19	0.16	0.18 ± 0.05
Sum	Bdx	−0.02	0.43	0.02	0.17	0.36	0.13	0.24		0.19 ± 0.06
	Roh	0.2	0.19	0.14	0.23	0.22	0.46	0.14	0.19	0.22 ± 0.04
		0.15 ± 0.04	0.21 ± 0.03	0.08 ± 0.03	0.17 ± 0.03	0.26 ± 0.04	0.25 ± 0.06	0.15 ± 0.03	0.12 ± 0.06	0.174 ± 0.022

Values are the intraclass correlation coefficients. For each temperature, mean values are calculated for the trident and segments A2–A4 excluding the sum

A2, A3, A4: abdominal pigmentation for tergites 2–4, Trid: thoracic pigmentation

Each coefficient is based on ten isofemale lines

darker in male than in female: this suggest that the bab expression is also somehow repressed in male A4. The pigmentation phenotype is, however, a consequence of complex interactions investigated here, namely the environment (temperature) and three kinds of genetic variables, that is segment position (a developmental constraint in a modular trait), sex and population geographic origin.

An antero-posterior gradient of phenotypic plasticity was previously observed in females (Gibert et al. 2000): average pigmentation, and also average plasticity, increase progressively toward the posterior end, from A2 to A7. Also the temperature of minimum pigmentation increases backward. We have made similar observations for male A2–A4. A comparison of the males of the two populations has confirmed what was already found in females, that is an average lighter pigmentation in the Indian population. An analysis of the within population genetic variability, with the isofemale line method, failed to reveal any significant difference between sexes and populations. The average intraclass correlation for segments A2–A4 is 0.22 ± 0.01 in females and 0.20 ± 0.02 in males. This value is significantly lower than that found in females for segments A5–A7 (David et al. 1990; Gibert et al. 1996).

A more detailed comparison of the sexes has evidenced, however, a diversity of significant differences, revealing the complexity of the interactions between the genetic cascade which is responsible of sex determination (Baker and Ridge 1980), with a possible specific role of *double-sex* (Kopp et al. 2000), and the other genetic systems which determine each segment and regulate pigment expression, and of course the thermal environment. Our analyses have considered specifically sexual dimorphism (SD) expressed at the level of each isofemale line, by the male–female (M–F) difference, or as a ratio. We found that SD decreased regularly with increasing temperature. At low temperatures, males are consistently darker than females and this difference is mainly due to A4. The sex difference disappears or is inverted, at temperatures higher than 25°C. This pattern is completely different for the body size SD, expressed as a F/M ratio, which increases at high temperatures (David et al. 1994).

We also found that the reactivity to temperature change was greater in males than in females, when measured by the curvature g_2 parameter of the reactions norms. While in female g_2 does not change among segments, it increases regularly in males from A2 to A4, reaching an almost double value. Interestingly, plasticity in male A5 is almost nil, because of the black pigmentation. If the expression of the *bab* gene is somehow inhibited in A4, this phenomenon is not strong enough to reduce the plasticity of this segment.

Our isofemale line design permitted to calculate a genetic correlation between female and male values (David et al. 2005). A correlation close to unity would imply a lack of genetic variability of SD (Roff 1992; Falconer and Mackay 1996). We found for all segments a positive correlation, but significantly less than 1. Interestingly the correlation for the thoracic pigmentation (0.75) was significantly higher than for the abdomen segments (0.49). This agrees with the fact that SD for the thorax pigmentation was always very low and on average close to zero (Fig. 4). In all cases, however, we found a significant heritability of SD. The overall value of the intraclass correlation (0.17) is significantly less than for the traits in each sex (0.22). Such a difference seems a general property of SD of various other traits, such as body size and bristle number (David et al. 2003, 2006b). The value significantly greater than zero seems however sufficient to permit an evolution by natural selection, when needed.

A final observation is that average pigmentations were not the same in the two populations compared. What has been found in males matches what was already known for the females (Gibert et al. 1998a): Indian males are on average lighter than French males, in agreement with the thermal adaptive hypothesis (see Introduction). More interestingly, we also found significant differences in SD. Up to know, there is only little information on the genetic variability of SD among populations. A recent investigation of body size and sternopleural bristle number in *D. melanogaster* and *Zaprionus indianus* (David et al. 2006b) failed to evidence any variation among geographic populations and it was suggested that SD might be submitted, in nature, to some strong stabilizing selection. Our results on pigmentation are at odds with observations on body size and a major question remains: can we consider the difference in SD pigmentation as an adaptation that is a consequence of different selective pressures on the two sexes? In this respect, our main observation is that, at high temperatures, French males become identical to the females, while Indian males become much lighter.

From that observation, an adaptive interpretation may be proposed. Within the frame of the thermal budget hypothesis, the permanent black pigmentation of A5–A6 in males should be a handicap at high temperature. Since these segments are unable to change, a stronger selection will be applied upon A2–A4, which are both genetically variable and plastic. This is exactly what has been observed, that is lighter males at high temperatures in the population living in a hot environment. Of course, a plausible hypothesis must be considered with caution and deserves more extensive investigations. Worth to recall is the fact that, in various tropical species of the melanogaster group (*Sophophora* subgenus) such as *D. kikkawai* (Gibert et al. 1999) or *D. santomea* (Llopart et al. 2002) males are completely yellow while females are polymorphic. Plasticity studies should be undertaken in several species which have dark abdomen males and which are able to live under

different climates. A difference in SD plasticity similar to what has been found here would be a strong argument in favor of an adaptive variation.

References

Andrewartha HG, Birch LC (1954) The distribution and abundance of animals. University of Chicago Press, Chicago

Bächli G (1971) Leucophenga und Paraleucophenga (Diptera Brachycera). Fondation pour favoriser les recherches scientifiques en Afrique, Bruxelles

Baker BS, Ridge KA (1980) Sex and the single cell I On the action of major loci affecting cell determination in *Drosophila melanogaster*. Genetics 94:383–423

Capy P, David JR, Robertson A (1988) Thoracic trident pigmentation in natural populations of *Drosophila simulans*: a comparison with *D. melanogaster*. Heredity 61:263–268. doi:10.1038/hdy.1988.114

Capy P, Pla E, David JR (1994) Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans*. II. Within-population variability. Genet Sel Evol 26:15–28. doi:10.1051/gse:19940102

Cossins AR, Bowler K (1987) Temperature biology of animals. Chapman and Hall, London and New York

David JR, Capy P (1988) Genetic variation of *Drosophila melanogaster* natural populations. Trends Genet 4:106–111

David JR, Clavel MF (1965) Interaction entre le génotype et le milieu d'élevage. Conséquences sur les caractéristiques du développement de la Drosophile. Bull Biol Fr Belg 99:369–378

David JR, Allemand R, Van Herrewege J, Cohet Y (1983) Ecophysiology: abiotic factors. In: Ashburner M, Carson HL, Thompson JN (eds) The genetics and biology of Drosophila. Academic Press, London, pp 105–170

David JR, Capy P, Payant V, Tsakas S (1985) Thoracic trident pigmentation in *Drosophila melanogaster*: differentiation of geographical populations. Genet Sel Evol 17:211–223. doi:10.1051/gse:19850204

David JR, Capy P, Gauthier JP (1990) Abdominal pigmentation and growth temperatures in *Drosophila melanogaster*: similarities and differences in the norms of reaction of successive segments. J Evol Biol 3:429–445. doi:10.1046/j.1420-9101.1990.3050429.x

David JR, Moreteau B, Gauthier JR, Pétavy G, Stockel J, Imasheva A (1994) Reaction norms of size characters in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis. Genet Sel Evol 26:229–251. doi:10.1051/gse:19940305

David JR, Gibert P, Gravot E, Pétavy G, Morin JP, Karan D et al (1997) Phenotypic plasticity and developmental temperature in Drosophila: analysis and significance of reaction norms of morphometrical traits. J Therm Biol 22:441–451. doi:10.1016/S0306-4565(97)00063-6

David J, Gibert P, Mignon-Grasteau S, Legout H, Pétavy G, Beaumont C et al (2003) Genetic variability of sexual size dimorphism in a natural population of *Drosophila melanogaster*: an isofemale-line approach. J Genet 82:79–88. doi:10.1007/BF02715810

David J, Gibert P, Legout H, Pétavy G, Capy P, Moreteau B (2005) Isofemale lines in Drosophila: an empirical approach to quantitative trait analysis in natural populations. Heredity 94:3–12. doi:10.1038/sj.hdy.6800562

David JR, Legout H, Moreteau B (2006a) Phenotypic plasticity of body size in a temperate population of *Drosophila melanogaster*: when the temperature-size rule does not apply. J Genet 85:9–23. doi:10.1007/BF02728965

David JR, Araripe LO, Bitner-Mathé BC, Capy P, Goni B, Klaczko LB et al (2006b) Sexual dimorphism of body size and sternopleural bristle number: a comparison of geographic populations of an invasive cosmopolitan drosophilid. Genetica 128:109–122. doi:10.1007/s10709-005-5539-5

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics. Prentice Hall, England

Gibert P, Moreteau B, Moreteau JC, David JR (1996) Growth temperature and adult pigmentation in two Drosophila sibling species: an adaptive convergence of reaction norms in sympatric populations? Evol Int J Org Evol 50:2346–2353. doi:10.2307/2410703

Gibert P, Moreteau B, Moreteau JC, Parkash R, David JR (1998a) Light body pigmentation in Indian *Drosophila melanogaster*: a likely adaptation to a hot and arid climate. J Genet 77:13–20

Gibert P, Moreteau B, Scheiner SM, David JR (1998b) Phenotypic plasticity of body pigmentation in Drosophila: correlated variations between segments. Genet Sel Evol 30:181–194. doi:10.1051/gse:19980207

Gibert P, Moreteau B, Munjal AK, David JR (1999) Phenotypic plasticity of abdominal pigmentation in D kikkawai: multiple interaction between major gene, sex, abdomen segment and growth temperature. Genetica 105:165–176. doi:10.1023/A:1003704315194

Gibert P, Moreteau B, David JR (2000) Developmental constraints on an adpative plasticity: reaction norms of pigmentation in adult segments of *Drosophila melanogaster*. Evol Dev 2:249–260. doi:10.1046/j.1525-142x.2000.00064.x

Gibert P, Capy P, Imasheva A, Moreteau B, Morin J, Pétavy G et al (2004a) Comparative analysis of morphometrical traits among *Drosophila melanogaster* and *D. simulans*: genetic variability, clines and phenotypic plasticity. Genetica 120:165–179. doi:10.1023/B:GENE.0000017639.62427.8b

Gibert P, Moreteau B, David JR (2004b) Phenotypic plasticity of body pigmentation in *Drosophila melanogaster*. Genetic repeatability of quantitative parameters in two successive generations. Heredity 92:499–507. doi:10.1038/sj.hdy.6800449

Gibert JM, Personnet F, Schlötterer C (2007) Phenotypic plasticity in Drosophila pigmentation caused by temperature sensitivity of a chromatin regualr network. PLoS Genet 3:e30. doi:10.1371/journal.pgen.0030030

Gibson AR, Falls JB (1979) Thermal biology of the common garter snake *Thamnophis sirtalis* (L.). II. The effects of melanism. Oecologia 43:99–109. doi:10.1007/BF00346675

Hoffmann AA, Parsons PA (1988) The analysis of quantitative variation in natural populations with isofemale strains. Genet Sel Evol 20:87–98. doi:10.1051/gse:19880108

Hoffman AA, Parsons PA (1991) Evolutionary genetics and environmental stress. Oxford University Press, Oxford

Hollocher H, Hatcher JL, Dyreson EG (2000) Evolution of abdominal pigmentation differences across species in the Drosophila dunni subgroup. Evol Int J Org Evol 54:2046–2056

Huey RB, Moreteau B, Moreteau JC, Gibert P, Gilchrist GW, Ives AR et al (2006) Sexual size dimorphism in a Drosophila clade, the D. obscura group. Zoology 109:318–330. doi:10.1016/j.zool.2006.04.003

Karan D, Morin JP, Gravot E, Moreteau B, David JR (1999) Body size reaction noms in *Drosophila melanogaster* temporal stability and genetic architecture in a natural population. Genet Sel Evol 31:491–508. doi:10.1051/gse:19990505

Kingsolver JG, Wiernasz DC (1991) Seasonal polyphenism in wing-melanin pattern and thermoregulatory adaptation in Pieris butterflies. Am Nat 137:816–830. doi:10.1086/285195

Klaczko LB (2006) Evolutionary genetics of *Drosophila mediopunctata*. Genetica 126:43–55. doi:10.1007/s10709-005-1431-6

Kopp A, Duncan I, Godt D, Carroll S (2000) Genetic control and evolution of sexually dimorphic characters in Drosophila. Nature 408:553–559. doi:10.1038/35046017

Kopp A, Graze R, Xu S, Carroll S, Nuzhdin S (2003) Quantitative trait loci responsible for variation in sexually dimorphic traits in *Drosophila melanogaster*. Genetics 163:771–787

Leather SR, Walters KFA, Bale JS (1993) The ecology of insect overwintering. Cambridge University Press, Cambridge

Llopart A, Elwyn S, Lachaise D, Coyne J (2002) Genetics of a difference in pigmentation between *Drosophila yakuba* and *Drosophila santomea*. Evol Int J Org Evol 56:2262–2277

Majerus MEN (1998) Melanism: evolution in action. Oxford Univ. Press, Oxford, UK

Moreteau B, Gibert P, Pétavy G, Moreteau JC, Huey RB, David JR (2003) Morphometrical evolution in a Drosophila clade: the Drosophila obscura group. J Zool Syst Evol Res 41:64–71. doi:10.1046/j.1439-0469.2003.00195.x

Munjal AK, Karan D, Gibert P, Moreteau B, Parkash R, David JR (1997) Thoracic trident pigmentation in *Drosophila melanogaster*: latitudinal and altitudinal clines in Indian populations. Genet Sel Evol 29:601–610. doi:10.1051/gse:19970505

Precht H, Christophersen J, Hensel H, Larcher W (1973) Temperature and life. Springer Verlag, Berlin

Reeve JP, Fairbairn DJ (1999) Change in sexual size dimorphism as a correlated response to selection on fecundity. Heredity 83:697–706. doi:10.1038/sj.hdy.6886160

Roff DA (1992) The evolution of life histories theory and analysis. Chapman & Hall, London

Rohmer C, David JR, Moreteau B, Joly D (2004) Heat induced male sterility in *Drosophila melanogaster*: adaptive genetic variations among geographic populations and role of the Y chromosome. J Exp Biol 207:2735–2743. doi:10.1242/jeb.01087

True JR (2003) Insect melanism: the molecules matter. Trends Ecol Evol 18:640–647. doi:10.1016/j.tree.2003.09.006

Via S (1984) The quantitative genetics of polyphagy in an insect herbivore II. Genetic correlations in larval performance within and among host plants. Evol Int J Org Evol 38:896–905. doi:10.2307/2408399

Watt WB (1969) Adaptive significance of pigment polymorphisms in Colias butterflies II. Thermoregulation and photoperiodically controlled melanin variation in Colias eurytheme. Proc Nat Acad Sci 63:767–774. doi:10.1073/pnas.63.3.767

Wittkop PJ, Carroll SB, Kopp A (2003) Evolution in black and white: genetic control of pigment patterns in Drosophila. Trends Genet 19:495–504. doi:10.1016/S0168-9525(03)00194-X