Genet. Res., Camb. (2003), **81**, *pp.* 25–32. *With 3 figures.* © 2003 Cambridge University Press
DOI: 10.1017/S0016672302005967 Printed in the United Kingdom

Phenotypic plasticity of sternopleural bristle number in temperate and tropical populations of *Drosophila melanogaster*

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(*Received 16 April 2002 and in revised form 29 August 2002*)

Summary

We investigated the phenotypic plasticity of sternopleural bristle (SB) number as a function of growth temperature in isofemale lines from temperate (France) and tropical (Congo) populations of *Drosophila melanogaster*. We found concave reaction norms with a maximum in the middle of the thermal range, except in four African lines which exhibited a regularly decreasing response curve. Genetic variability (intraclass correlation) and evolvability (genetic CV, coefficient of variation) were independent properties and did not change with temperature. Residual, within-line variability was, however, strongly influenced by growth temperature, showing a U-shaped response curve and a minimum CV of 9% at 21·5 °C. As expected from a previously known latitudinal cline, maximum values (MV) were higher in temperate than in tropical flies. The temperature of maximum value (TMV) was observed at a higher temperature in the tropical population, in agreement with similar adaptive trends already observed for other quantitative traits. Significant negative correlations within each population were observed between a plasticity curvature parameter and MV or TMV. No difference in curvature was, however, observed between populations, in spite of their very different MVs.

1. Introduction

Sternopleural bristle (SB) number is a classical trait for quantitative genetic studies in *Drosophila melanogaster*, showing a high heritability and a rapid response to directional selection (Falconer & Mackay, 1996; Mackay, 1996; Bubliy *et al*., 2000). More recently it became a classic model for quantitative trait locus (QTL) research (Long *et al*., 1996, 2000; Nuzhdin *et al*., 1998, 1999). The fact that SB number exhibits latitudinal clinal variation (Capy *et al*., 1993) suggests that it is related to fitness and under natural selection even if the target of selection is not clear. Moreover, since latitude is always strongly correlated with temperature, it can be assumed that ambient temperature is the responsible selective force. For example, if we consider the thermal conditions prevailing at both ends of a cline between Africa and Europe, average year temperature is about 26 °C at the equator but less than 15 °C in France.

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Besides its genetic variability and its likely response to natural selection, SB number is, like other quantitative characters (David *et al*., 1994; Delpuech *et al*., 1995; Noach *et al*., 1996; James *et al*., 1997), a plastic trait, the mean of which changes according to developmental temperature. The response curve of a quantitative trait to an environmental gradient is called the reaction norm. Polynomial adjustments are a convenient means for describing the norms and calculating characteristic values such as the coordinates of a maximum (see David *et al*., 1997; Gibert *et al*., 1998*a*). It was recently shown (Morin *et al*., 1999), by comparing tropical and temperate populations of *D. melanogaster* and *D. simulans*, that the reaction norms of size-related traits exhibited differences in their shape, which were probably an adaptation to local climates. More precisely, maximum size was observed at a higher temperature in warm-adapted populations. Analogous, but larger changes were also observed when comparing different species (Moreteau *et al*., 1997; Morin *et al*., 1997; Karan *et al*., 1999*b*). In this study, we asked three related questions: (1) What is the precise

shape of the reaction norm of SB number in relation to growth temperature in *D. melanogaster*? (2) Are there significant differences between equatorial African and temperate European populations? (3) Are the results in *D. melanogaster* similar to those already found in a distantly related species, *Zaprionus indianus* (Karan *et al.*, 1999*b*)?

We find that reaction norms can be described by quadratic polynomial with a maximum in the middle of the thermal range and a shape similar to that found in ovariole number (Delpuech *et al.*, 1995). A highly significant difference in SB number exists between equatorial and temperate populations, in agreement with the previously identified latitudinal clines, and also a slight difference in the shape of reaction norm.

2. Materials and methods

(i) Drosophila *populations*

We investigated three natural populations of *D. melanogaster*: a temperate one collected near Bordeaux (France) and two Afrotropical ones collected simultaneously in and near the Brazzaville area (Congo). In this area, one population was caught within the city (Kronenbourg brewery) while the other came from the countryside (Loukanga). These two types of populations are known to exhibit major genetic differences with respect to their alcohol tolerance and related enzyme loci (see Capy *et al.*, 2000).

(ii) *Experiments*

Wild-living females from each population were isolated in culture vials to initiate about 20 isofemale lines. The lines were kept for a few months in the laboratory at 20 °C, that is a maximum of six generations. Then 10 lines were randomly taken to start the experiments. From each line, 10 pairs of adults were taken as parents of the experimental flies. Each parental group was allowed to lay eggs for a few hours at 20–21 °C, on a killed-yeast, high-nutrient medium. With that procedure, population density per vial ranged between 80 and 150 adult flies. The use of a high-nutrient food prevents any crowding effect (see Karan *et al.*, 1999*a*). After egg-laying, vials were transferred to constant-temperature incubators until adult emergence. For the temperate population, we used seven temperatures (12, 14, 17, 21, 25, 28, 31 °C). For the two tropical populations, viability at 12 °C was low and this temperature could not be used in our experimental scheme. After emergence, adults were transferred to a middle temperature (around 20 °C) for a few days. Sternopleural bristles were counted, on females only, on both sides of the thorax. The total number of bristles was used as the variable in all calculations except when fluctuating asymmetry (FA) was considered. For each temperature and line, 10 females were randomly taken and measured. The whole data set is based on 2100 flies.

(iii) *Data analysis*

Data were analysed with Statistica software (1999). For each isofemale line, the response curve (reaction norm) to growth temperature was adjusted to a quadratic polynomial according to the equation:

$$\text{SB number} = \text{MV} + g_2(\text{t} - \text{TMV})^2,$$

where MV is the maximum value, TMV is the temperature of maximum value, t is temperature and g_2 a polynomial coefficient (curvature). Each line is thus defined by three characteristic values: the coordinates of the maximum and the curvature parameter (see David *et al.*, 1997). For measuring fluctuating asymmetry (FA) we used a relative estimate as in a previous work (Delpuech *et al.*, 1995):

$$\text{FA} = 2|\text{L} - \text{R}|/\text{L} + \text{R},$$

where L and R indicate numbers on left and right side. This value was multiplied by 100 so that the magnitude of FA is similar to that of a CV.

3. Results

(i) *Average response curves: reaction norms*

For a general overview of the shape of the reaction norms (SB number as a function of growth temperature in each population), the average response curves were considered and adjusted to a quadratic polynomial (Fig. 1*A*). In each case, a convenient adjustment was obtained (R^2 values of 0·94, 0·89 and 0·90 for Bordeaux, Kronenbourg and Loukanga respectively). The shapes are similar: reaction norms are concave, bowed downwards curves with a maximum in the middle of the thermal range, at 19·8, 20·4 and 21·2 °C for Bordeaux, Kronenbourg and Loukanga respectively. The SB number was always significantly higher in the temperate, Bordeaux population, than in the two African ones (Student's *t*-tests, not shown). This agrees with the known latitudinal cline for this trait (Capy *et al.*, 1993).

The regularity which is seen in the average curves encompasses a large variability among lines (Fig. 1*B*). For example, in the African populations, some lines exhibit high SB numbers, up to 19, while others do not exceed 17. ANOVA applied to each population (not shown) revealed highly significant effects of temperature and lines. A significant line × temperature interaction, although less pronounced, indicated that the reaction norms are not parallel and exhibit slightly different shapes.

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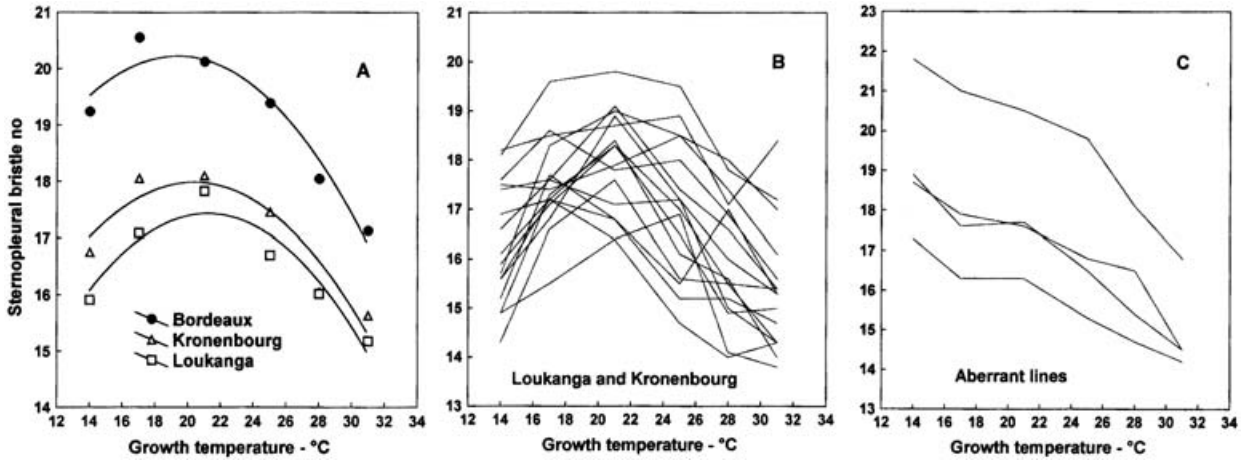

Fig. 1. Reaction norms of sternopleural bristle number in natural populations of *D. melanogaster* from France (Bordeaux) and Congo (Kronenbourg and Loukanga). (*A*) Average reaction norms in the three populations (quadratic adjustment). (*B*) Isofemale lines reaction norms in the two African populations (16 lines showing a maximum within the viable thermal range are shown). (*C*) Reaction norms of four 'aberrant' lines showing a regular decrease in bristle number with increasing growth temperature.

(ii) *Environmental variability and fluctuating asymmetry*

With an isofemale line experimental design, the within-line variance is often considered as an approximate estimate of the environmental variance (Noach *et al*., 1996; Bell, 1997; Imasheva *et al*., 2000) and, for comparing traits with different mean values, the within-line coefficient of variation is generally used (David *et al*., 1994). We found significant variations in the CVs according to developmental temperature, but very similar results in the three populations (Fig. 2) (ANOVA, not shown). In each case, high values were observed at extreme temperatures and minimal values in the middle of the thermal range. Such average curves can also be considered as quadratic convex reaction norms and the temperatures of minimum value were calculated to be 21·5 °C, 21·5 °C and 21·6 °C in Bordeaux, Kronenbourg and Loukanga respectively, i.e. very close to the physiological optimum of *D. melanogaster* (see Pétavy *et al*., 2001).

Fig. 2 suggests a better developmental homeostasis under optimal conditions. Another way to analyse developmental stability is to consider FA (Møller & Swaddle, 1997). We used a relative measure (see Section 2) and FA was calculated for each fly. Results were far less regular than for the CVs. Indeed, ANOVA applied to these data failed to show any significant difference due to line, population and temperature. It was quite clear, however, that when FA was expressed as a percentage, its overall value was about 11 %, and close to the mean of the within-line CV. This suggests that FA and CV might express similar responses to environmental fluctuations. For each line, we calculated over temperatures an average CV and an average FA: these two values were, however, completely independent ($r = 0{\cdot}08$, $n = 30$).

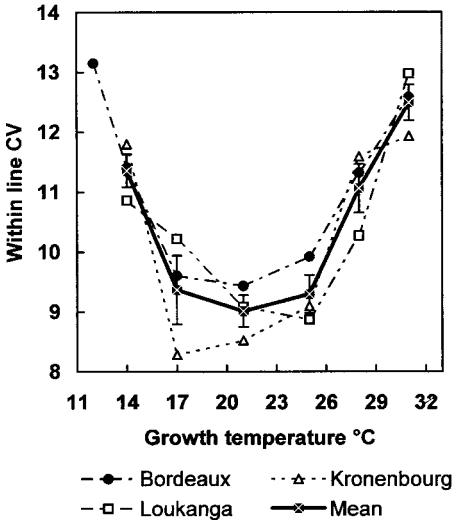

Fig. 2. Relationship between growth temperature and the within-line coefficient of variation of sternopleural bristle number. Vertical bars indicate the standard error.

(iii) *Genetic variability of SB number: intraclass correlation and evolvability*

Significant variation between isofemale lines indicates segregating genetic variability of the trait. This is used for calculating an intraclass correlation, sometimes referred to as a broad sense heritability, or isofemale line heritability (Parsons, 1983; Capy *et al*., 1993; Roff, 1998). Values (Table 1) ranged between 0·08 and 0·43, with an average of 0·30. Over the three populations, the maximum genetic variability was observed at 25 °C, but the temperature effect was not significant (ANOVA, not shown).

The capacity for a quantitative trait to change under selection depends not only on its heritability but also on its mean value, and this property has been defined as

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Table 1. *Intraclass correlation coefficients* (*isofemale line heritabilities*) *and evolvability* (*genetic CV*) *in the three investigated populations in relation to growth temperature*

	12 °C	14 °C	17 °C	21 °C	25 °C	28 °C	31 °C	Mean	SE
Bordeaux									
t	0·31	0·30	0·32	0·29	0·43	0·32	0·17	0·31	0·03
CVg	8·91	7·75	6·86	6·09	9·57	8·25	5·81	7·39	0·54
Loukanga									
t		0·19	0·08	0·27	0·42	0·35	0·14	0·24	0·05
CVg		10·8	5·98	5·72	7·83	6·61	10·98	7·99	0·88
Kronenbourg									
t		0·43	0·32	0·31	0·39	0·24	0·38	0·35	0·03
CVg		5·42	3·1	5·73	7·54	7·93	5·22	5·82	0·65
Mean									
t		0·31	0·24	0·29	0·41	0·30	0·23	0·30	0·03
SE		(0·07)	(0·08)	(0·01)	(0·01)	(0·03)	(0·08)		
CVg		7·99	5·31	5·85	8·31	7·60	7·34	7·07	0·49
SE		(1·56)	(1·14)	(0·12)	(0·63)	(0·50)	(1·83)		

t, intraclass correlation; CVg, evolvability; SE, standard error.

evolvability (Houle, 1992). Evolvability can be estimated by the genetic CV, that is the ratio of the square root of the genetic variance to the mean, and values are also given in Table 1. Evolvabilities (on average 7·07) were not significantly different between either populations or temperatures (ANOVA, not shown). Both the intraclass correlation and evolvability harbour the genetic variance at the numerator, and it might be argued that this could induce a spurious positive correlation. We calculated the correlation between values given in Table 1 and found a very low, non-significant overall correlation ($r = -0{\cdot}17$, $P = 0{\cdot}47$). Clearly, as for size-related traits (Karan *et al*., 1999*a*), heritability and evolvability provide independent genetic information.

(iv) *Genetic variability of the shape of reaction norms*

For investigating the genetic variability of reaction norms, each line was adjusted to a quadratic polynomial and then characteristic values were calculated (see David *et al*., 1997). For a quadratic polynomial, we calculated the coordinates of the maximum, that is the maximum value (MV) of bristle number and the temperature of this maximum (TMV). Also the g_2 polynomial coefficient provides information on the curvature of each norm.

Adjustments led to an unexpected result. For the 10 Bordeaux lines it was possible to calculate a maximum value within the experimental thermal range (12–31 °C). Among the 20 African lines, this was, however, not always possible. We considered the distribution of TMVs in the whole sample of 30 lines and found that 26 of them followed a unimodal distribution with a mean of $20{\cdot}23 \pm 0{\cdot}32$ °C (range 15·3–22·6 °C). The four others were quite apart from this main distribution, with TMVs below 12 °C, that is more than four standard deviations from the mean. Three of these aberrant lines were found in Kronenbourg and one in the Loukanga population. The reaction norms of the two sets of African lines are shown Fig. 1*B* and *C*: 16 lines exhibit concave response curves with a maximum within the thermal range, also found in Bordeaux. The four aberrant lines, on the other hand, show a regular decreasing trend between 14 and 31 °C.

For further calculations (Table 2) and a comparison between geographic populations, we considered only the 16 'normal' African lines. As expected, average maximum values differed between the French (20·3) and the African lines (17·7). TMVs were observed at a lower temperature (19·50 °C) in the temperate population than in the equatorial African ones (20·7 °C) but the difference, 1·2 °C, is non-significant (Table 2). However, the difference becomes significant ($P = 0{\cdot}047$) if we compare the Bordeaux and Loukanga populations. Such a comparison is justified since the Loukanga sample corresponds to a field African population, while the brewery race (Kronenbourg) is likely to have been introgressed by a European propagule in the recent past (Capy *et al*., 2000). Differences between curvatures were non-significant. The goodness of fit of polynomial adjustments was not very good with average R^2 slightly above 0·70. This is due to irregularities in the shapes of individual curves, as shown in Fig. 1*B*. A cubic polynomial adjustment failed to improve the R^2 values.

Among the three characteristic values given in Table 2, one concerns the trait itself (MV) while the two others (TMV and g_2) are plasticity parameters and characterize the reactivity of each line to growth temperature. We calculated the genetic correlations between these three characteristics. For increasing the precision of the analysis, we considered the total set of 26 quadratic norms. Since the mean MVs are different

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Table 2. *Characteristic values of the reaction norms of the 'normal' lines in the three investigated populations*

	Bordeaux ($n=10$)	Kronenbourg ($n=7$)	Loukanga ($n=9$)	ANOVA F	ANOVA P
MV					
m	20·3 ± 0·5	18·0 ± 0·4	17·5 ± 0·4	13·16	< 0·001
CV	7·9	5·4	6·0		
TMV					
m	19·5 ± 0·6	20·1 ± 0·5	21·1 ± 0·5	2·59	0·096
CV	9·6	6·3	6·5		
g_2					
m	−0·027 ± 0·003	−0·024 ± 0·004	−0·026 ± 0·004	0·16	0·849
CV	39·4	44·3	41·2		
R^2					
m	0·74 ± 0·06	0·69 ± 0·10	0·70 ± 0·07	0·07	0·931
CV	25·7	41·4	30·7		

n, number of lines; MV, maximum value; TMV, temperature of maximum value; g_2, curvature parameter; R^2, adjusted R^2; *m*, mean value ± SE; CV, coefficient of variation among lines; ANOVA, one-way analysis of differences among populations; *F*, variance ratio; *P*, significance probability.

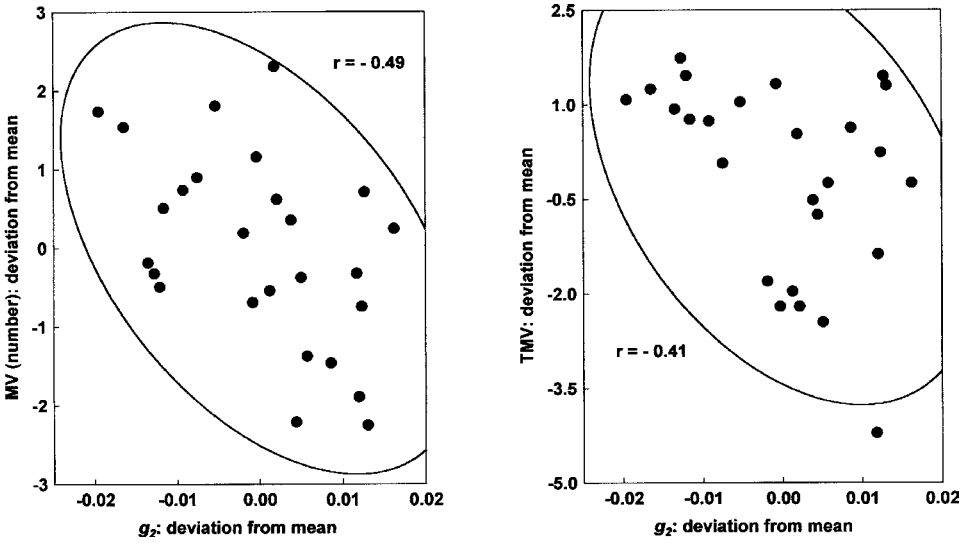

Fig. 3. Negative correlations between the g_2 curvature parameter of each isofemale line and either the maximum value of bristles (MV, left-hand graph) or the temperature of maximum value (TMV, right-hand graph). Each point corresponds to an isofemale line, and the deviation from the mean value of each population is considered for each line. Ellipses of 0·90 probabilities are shown.

between populations, we characterized each line by the deviation from the mean of its population. The same transformation was also applied to TMVs and g_2.

We found a significant overall negative correlation between MV and g_2 ($r=-0{\cdot}49$, $P=0{\cdot}012$; Fig. 3), which means a stronger curvature in lines with a higher SB number. Also a negative correlation between the two plasticity characteristics (g_2 and TMV) was observed ($r=-0{\cdot}41$, $P=0{\cdot}037$; Fig. 3). However, the correlation (not shown) between MV and TMV ($r=0{\cdot}13$) was non-significant.

4. Discussion

The lines investigated were kept for a few generations under laboratory conditions before being investigated for their thermal phenotypic plasticity. One may wonder whether this delay could have resulted in some kind of laboratory adaptation, changing their average phenotype and possibly also their phenotypic plasticity. Specific studies should be undertaken to address this problem precisely, but our present knowledge suggests such was not the case. Firstly, it is known that

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rearing the same strain at different temperatures may result in some divergence in body size, but several years are necessary for a significant change (Cavicchi *et al.*, 1985). Second, we still observed significant differences between European and Afrotropical lines, in agreement with an already known latitudinal cline (Capy *et al.*, 1993). Finally, an investigation of isofemale lines over nine laboratory generations evidenced a good phenotypic stability of each line over time (Gibert *et al.*, 1998*c*). This study concerned wing length and abdomen pigmentation, but we may suggest that this stability was also valid for SB bristles.

Although genetically variable, SB number is also a plastic trait, responding to developmental temperature. Up to now, only a few investigations have paid attention to this plasticity (Gurganus *et al.*, 1998; Mackay & Lyman, 1998), using two or three different temperatures only. Our extensive investigations on three different populations have clearly shown that the average reaction norms are concave curves with a maximum (optimum?) around 20–21 °C.

The within-line variability, which mainly expresses an environmental component, exhibited a clear-cut minimum at medium temperatures and significant increased at extreme temperatures. Such a phenomenon has been found previously for body size traits (David *et al.*, 1994; Noach *et al.*, 1996; Karan *et al.*, 1998*a*, 1999*a*; Imasheva *et al.*, 2000) and might be a general result in *D. melanogaster*. The general interpretation is that extreme temperatures, low or cold, are stressful and decrease the stability of developmental processes (Bijlsma & Loeschcke, 1997; Hoffmann & Parsons, 1997). Another way of investigating developmental stability is to consider fluctuating asymmetry (FA). FA was high for SB number but we failed to find any significant variation according to growth temperature, while such a phenomenon was found for ovariole number (Delpuech *et al.*, 1995) and also for SB number in another investigation (Bubliy *et al.*, 2000).

Genetic variability among lines, estimated by the intraclass correlation, was similar in the three populations investigated and did not change significantly with temperature. Evolvability (Houle, 1992) estimated by the genetic CV was also not influenced by growth temperature or population origin. Interestingly, as for size-related traits (Karan *et al.*, 1999*a*), heritability and evolvability appear to be independent genetic properties.

Among 30 isofemale line reaction norms, 26 could be conveniently adjusted to a quadratic concave polynomial, allowing us to calculate three characteristic values (David *et al.*, 1997). One of them, the maximum value (MV), is a trait attribute. The two others define the plasticity of the trait: TMV (temperature of maximum value) gives the position of the norm along the temperature axis while g_2 is a curvature parameter. As expected from the latitudinal cline, MVs were very different between populations. The average curvatures of the norm (g_2) were, however, similar. Concerning TMVs we know, for other traits, that higher values are expected in warm-adapted species (Moreteau *et al.*, 1997; Morin *et al.*, 1997; Karan *et al.*, 1999*b*) and in geographic populations living in tropical habitats (Delpuech *et al.*, 1995; Morin *et al.*, 1999). Our results did fit this expectation, since a difference of 1·6 °C was observed between the Bordeaux and Loukanga populations. It is of note that significant differences in TMVs of other traits between tropical and temperate populations of *D. melanogaster*, even when significant, were never very large (Morin *et al.*, 1999), contrasting with differences sometimes exceeding 10 °C among different species (Moreteau *et al.*, 1997; Morin *et al.*, 1997).

Genetic correlations among characteristic values of the reaction norms are usually low and non-significant (Delpuech *et al.*, 1995), in agreement with the general idea that plasticity and trait value are genetically independent (Scheiner, 1993). For sternopleural bristles, we found, however, a significant correlation between the curvature parameter g_2 and two other traits, MV and TMV. More precisely, a stronger reactivity to temperature within a population is associated with a higher trait value and a higher TMV. Whether this applies to other traits remains to be investigated.

Up to now, the phenotypic plasticity of SB number over the complete thermal range of a species has been investigated in only one drosophilid, the tropical *Zaprionus indianus* (Karan *et al.*, 1999*b*). Results in *D. melanogaster* revealed similarities but also some differences. In both species, concave reaction norms were found with a maximum within the thermal range, but the polynomial adjustments were not very good (average $R^2 < 0{\cdot}80$ in both cases). Indeed aberrant lines, impossible to adjust to a quadratic polynomial, were also observed in *Zaprionus*. *Z. indianus* is a purely tropical, warm-adapted species and we expected higher TMVs than in *D. melanogaster*. Indeed, this prediction was confirmed for wing and thorax length and ovariole number, but not for SB (Karan *et al.*, 1999*b*). For this trait, TMV was 19·0 °C, against 21·1 °C in the Afrotropical *D. melanogaster*.

Finding latitudinal clines for quantitative characters is always a powerful argument for assuming that the relevant traits are related to fitness (Capy *et al.*, 1993; Karan *et al.*, 1998*b*, 1999*b*; James *et al.*, 1997; Azevedo *et al.*, 1998; Zwaan *et al.*, 2000). However, such empirical observations raise two kinds of questions: (1) To what extent is a cline a correlated response of natural selection acting on another trait? (2) If the trait considered is a direct target for natural selection, what is its relationship with fitness?

Concerning SB number, it might be argued that the cline is explained by the latitudinal cline of body

size: a bigger thorax would leave more space for harbouring more bristles. To be valid, however, this interpretation implies a positive genetic correlation with thorax size. Previous data obtained on populations from the same geographic area (David *et al*., 1977) produced a slight, non-significant correlation ($r = 0{\cdot}18$). We also have data (unpublished results) obtained with the isofemale line method and pointing to a lack of genetic correlation. The most likely interpretation is that SB number is a trait directly affected by natural selection. An indirect argument, suggested in several papers (Kearsey & Barnes, 1970; Mackay *et al*., 1995; Nuzhdin *et al*., 1995), is that SB number is under strong balancing selection. SB are sensory bristles (Mackay, 1996), but this sensory function might not be the direct target for selection. Numerous genes are progressively discovered to be quantitative trait loci for SB but also to have other roles during development (Nuzhdin *et al*., 1999). Indeed Kearsey & Barnes (1970) showed that larval density was acting selectively upon SB number, and of course, independently of the adult phenotype. As stated by T. Mackay (personal communication) 'the bulk of evidence for sternopleural bristles favors selection on a trait pleiotropically connected to fitness'.

Further investigations are needed to establish whether thermal regimes are able to change the average SB number and also the shape of the reaction norms of this phenotype. It would be very interesting to identify the quantitative trait loci which discriminate tropical and temperate populations.

We thank Charles H. Langley and Trudy F. C. Mackay for helpful comments on an earlier draft of this paper.

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