RESEARCH ARTICLE

Phenotypic plasticity of body size in a temperate population of *Drosophila melanogaster*: when the temperature–size rule does not apply

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Abstract

A natural population of *Drosophila melanogaster* in southern France was sampled in three different years and 10 isofemale lines were investigated from each sample. Two size-related traits, wing and thorax length, were measured and the wing/thorax ratio was also calculated. Phenotypic plasticity was analysed after development at seven different constant temperatures, ranging from 12°C to 31°C. The three year samples exhibited similar reaction norms, suggesting a stable genetic architecture in the natural population. The whole sample (30 lines) was used to determine precisely the shape of each reaction norm, using a derivative analysis. The practical conclusion was that polynomial adjustments could be used in all cases, but with different degrees: linear for the wing/thorax ratio, quadratic for thorax length, and cubic for wing length. Both wing and thorax length exhibited concave reaction norms, with a maximum within the viable thermal range. The temperatures of the maxima were, however, quite different, around 15°C for the wing and 19.5°C for the thorax. Assuming that thorax length is a better estimate of body size, it is not possible to state that increasing the temperature results in monotonically decreasing size (the temperature–size rule), although this is often seen to be the case for genetic variations in latitudinal clines. The variability of the traits was investigated at two levels—within and between lines—and expressed as a coefficient of variation. The within-line (environmental) variability revealed a regular, quadratic convex reaction norm for the three traits, with a minimum around 21°C. This temperature of minimum variability may be considered as a physiological optimum, while extreme temperatures are stressful. The between-line (genetic) variability could also be adjusted to quadratic polynomials, but the curvature parameters were not significant. Our results show that the mean values of the traits and their variance are both plastic, but react in different ways along a temperature gradient. Extreme low or high temperatures decrease the size but increase the variability. These effects may be considered as a functional response to environmental stress.

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Introduction

The comparison of animal species across different kingdoms reveals huge variations in body size, and evolutionists have long been fascinated by such variations. Size is either considered as a cause, responsible for changes in the proportions of various organs due to allometric relationships, or as an effect determined by an adaptation to different ecological niches (Peters 1983; Reiss 1989; Harvey and Pagel 1991; Blanckenhorn 2000).

In the drosophilid family, size variations between species are not large, restricted to about one order of magnitude (range 0.5–5 mg; G. Pétavy, personal communication.). How such variations influence the overall shape and proportions of body parts is not documented. There is, however, more information on microevolutionary variations, generally related to climate. In this respect, latitudinal clines have been found in almost all species investigated, including *Drosophila robusta* (Stalker and Carson 1947), *D. subobscura* (Prevosti 1955; Misra and Reeve 1964), *D. melanogaster* and *D. simulans* (Capy *et al*. 1993; Gilchrist and Partridge 1999; Gibert *et al*. 2004a), *D. virilis* (David and Kitagawa 1982), *D. kikkawai*

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(Karan *et al*. 1998) and *Zaprionus indianus* (Karan *et al*. 2000). In all these cases, the same trend has been observed, i.e. an increase of size toward higher latitudes and hence colder places, suggesting a general climatic adaptation.

In *Drosophila*, genetic variations revealing a cline are generally demonstrated under standard laboratory conditions, which minimize the environmental variance. In nature, however, wild-collected flies show a large phenotypic variance in size, implying a low heritability of body size (Coyne and Beecham 1987; Imasheva *et al*. 1997; Gibert *et al*. 1998a). We are aware of only a few studies in which size was investigated, among geographic populations, both in nature and in the laboratory (Coyne and Beecham 1987; Imasheva *et al*. 1994; James *et al*. 1997).

Phenotypic variability in nature has two main origins: differences in larval feeding conditions and temperature variations across microhabitats. Most investigations on phenotypic plasticity of size have considered the role of temperature, with the hope of understanding latitudinal clines. The results have revealed an overall parallelism between plasticity and clines (Atkinson 1994; Atkinson and Sibly 1997). In both cases, a decrease in size occurs as a consequence of higher temperature, either within one generation (plasticity) or after more than 100 generations (Cavicchi *et al*. 1985; Van Voorhies 1996; Huey *et al*. 2000). The plastic effect is often called the temperature–size rule, which implies that a temperature increase will result in a smaller adult size of ectotherms. A diversity of physiological adaptive or nonadaptive mechanisms may account for this phenomenon (van der Have and de Jong 1996; Gibert and de Jong 2001; Angilletta and Dunham 2003). The genetic clines are akin to Bergmann's rule for homeotherms (Bergmann 1847), but the increase of size observed at higher latitudes is not found in all ectotherm species (Blanckenhorn and Demont 2004). When similar latitudinal clines are observed, such as in *Drosophila*, there is no consensus interpretation for such adaptive variations: we still do not clearly understand why it is better to be bigger in the cold (Partridge and Coyne 1997; Pétavy *et al*. 1997; Bochdanovits and de Jong 2003a,b; Angilletta *et al*. 2004). Finally, previous investigations in *Drosophila* have unravelled a new complication, since the response curves of size traits to growth temperature (the reaction norms) are generally not linear, but rather bowed downward (David *et al*. 1994, 1997, 2004; Karan *et al*. 1999). The assumption that overall size decreases monotonically with increasing temperature appears to be an oversimplification. When considering genetic clinal variations, a linear adjustment according to latitude (or temperature) appears to be the best fit. However, there is usually a large dispersal around the regression, which might be due to significant differences among local populations, but also to laboratory drift (Capy *et al*. 1993; Gibert *et al*. 2004a).

In this context, the present work was undertaken for several complementary purposes: first, to compare samples taken from the same natural population, but in different years, and thus analyse the genetic stability of a local population not only for its size, but also for its plasticity; second, to provide a precise comparison of the shapes of the reaction norms of the two most investigated size-related traits, namely wing length and thorax length; third, to analyse how variability of the traits may change according to growth temperature.

We show that, for the three traits investigated here (wing and thorax length, and wing/thorax (W/T) ratio), polynomial adjustments are convenient descriptors of the reaction norms, but that different degrees (linear, quadratic or cubic) should be implemented according to the trait, and that there is a substantial difference between the behaviours of wing and thorax length under this technique. By contrast, the phenotypic variability of all traits exhibits a specific, convex quadratic norm, with a minimum at an intermediate temperature which might be a physiological optimum.

Materials and methods

Wild *D. melanogaster* adults were collected in the Grande Ferrade estate (Institut National de la Recherche Agronomique, INRA) near Bordeaux (southern France). Collections were always made at the end of the year, in November, and larval breeding sites were decaying grapes in a vineyard. Adults were captured either with a net or banana baits. Three independent samples were collected in 1992, 1997 and 1999. Adults were brought to the laboratory and pairs (one female and one male) were randomly established and set in culture vials at 20–22°C. These wild-caught adults were kept in vials for 3–4 days and then discarded. After progeny emergence, 10 females and 10 males from each line were randomly taken as parents of the experimental flies. After a few days, these parents were transferred to culture vials containing a high nutrient medium (8% dried yeast; David and Clavel 1965). Oviposition took place at 20–22°C, lasted about 6 h, and the operation was repeated seven times. Immediately after removing the parents, each batch of culture vials was transferred to one of seven experimental temperatures, namely 12, 14, 17, 21, 25, 28 and 31°C. The temperature regulation was ±0.1°C, thanks to the fact that the incubators were themselves kept in a regulated room, at 20°C for the incubators at 25°C and above, and in another room regulated at 6–8°C for temperatures of 12, 14 and 17°C. The temperature of 21°C was obtained in a third climatic room, which was less precisely regulated (±0.5°C). The larval density was not precisely controlled and ranged between 100 and 200 per vial. Such fluctuations do not influence adult body size, owing to the high amount of yeast in the rearing medium. In a previous study it was found that body size remained constant when the larval density increased from 20 to 320 in the same vial (Karan *et al*. 1999). After emergence, the adults were transferred to fresh food, aged for a few days at 21°C, and then measured after ether anaesthesia with a binocular microscope. Two measurements were taken on each fly and micrometer units transformed into mm × 100. Thorax length

was measured on a left-side view, from the neck to the tip of the scutellum, as done in most studies analysing this trait. Total wing length was measured from its thoracic articulation to the tip. This is quite unusual; in most published works, the wing is removed, mounted on a microscope slide, and the length is estimated between specific landmarks, quite variable among authors. We prefer our method, which measures the whole mobile part of the wing. This also helps to estimate the wing loading, as the inverse of the W/T ratio (Pétavy *et al*. 1997).

For each year's sample, we investigated 10 lines, and for each line 10 females and 10 males were measured. Thus, the total data set is made up of 30 lines, measured at seven constant temperatures, for a total number of 4200 flies.

Data were analysed in a diversity of ways, which will be described precisely in the Results section. Variations of trait values according to growth temperature (the reaction norms) were analysed with polynomial adjustments (David *et al*. 1997, 2004). With our experimental design, the variability among flies was analysed at two levels: within and between lines, providing information on environmental and genetic variability respectively. Because of a scaling effect, we considered in most cases the coefficient of variation (CV).

All calculations were done with Statistica version 5.5 software (StatSoft 1999).

Results

Analysis of mean values

Comparison of the three temporal samples: The overall mean values of wing and thorax length and of the W/T ratio are given in table 1. The results of the three year samples were subjected to analysis of variance (ANOVA) (table 2). As expected, a main effect of temperature was always detected, and also a highly significant variation between lines of each sample. The main effect of years was never significant, while the year × temperature interaction was always highly significant, indicating that the variability of size traits according to growth temperature was not exactly the same in the different years.

ANOVA is a powerful statistical means for unravelling significant variations, especially in big samples. So we did not consider the *F* values, but the magnitude of each effect, that is the amount of the total variance explained in each case (table 2). We see the preponderance of the temperature effect, which on average explains more than 80% of the variability. By contrast, the year × temperature interaction, despite its significance, accounts for less than 1%. The line × temperature interaction explains 2.5% of the total variability, and this effect might be due to the random sampling of the lines in different years. Finally the error term, that is

Table 1. Mean values (± s.e.) of size traits according to growth temperature. Mean values of 30 isofemale lines from three different years are pooled and used in statistical calculations. Wing and thorax are expressed in mm × 100.

Trait	Sex	12°C	14°C	17°C	21°C	25°C	28°C	31°C
Wing length	♀	289.1 ± 1.5	299.7 ± 1.2	299.5 ± 1.1	283.8 ± 1.3	270.8 ± 1.0	257.7 ± 0.9	239.9 ± 1.0
	♂	261.6 ± 1.2	271.0 ± 1.1	267.8 ± 1.1	248.6 ± 1.4	233.4 ± 0.9	221.8 ± 0.7	206.9 ± 0.8
Thorax length	♀	104.1 ± 0.4	109.1 ± 0.4	111.6 ± 0.3	111.3 ± 0.3	109.0 ± 0.3	106.0 ± 0.3	99.8 ± 0.4
	♂	93.7 ± 0.4	98.7 ± 0.3	100.1 ± 0.3	98.7 ± 0.3	94.9 ± 0.3	91.3 ± 0.3	86.0 ± 0.3
W/T ratio	♀	2.779 ± 0.007	2.747 ± 0.006	2.683 ± 0.006	2.549 ± 0.008	2.484 ± 0.006	2.431 ± 0.005	2.406 ± 0.005
	♂	2.791 ± 0.006	2.746 ± 0.007	2.674 ± 0.007	2.519 ± 0.009	2.460 ± 0.006	2.429 ± 0.005	2.407 ± 0.006

Table 2. Results of ANOVA on wing and thorax length and W/T ratio. The table gives the proportion of the total variance explained by the different factors.

		Wing length		Thorax length		W/T ratio	
Source of variation	df	Female	Male	Female	Male	Female	Male
(1) Year	2	0.60^{ns}	0.60^{ns}	0.46	0.61^{ns}	0.25	0.23^{ns}
(2) Temperature	6	85.58^{***}	89.44^{***}	63.93^{***}	75.40^{***}	86.97^{***}	87.85^{***}
(3) Line (year)	27	4.64^{***}	2.94^{***}	6.92^{***}	4.08^{***}	2.73^{***}	2.38^{***}
1 × 2	12	0.89^{***}	0.77^{***}	1.66^{***}	1.21^{***}	0.47^{***}	0.65^{***}
2 × 3	162	1.68^{***}	1.35^{***}	5.04^{***}	3.55^{***}	1.78^{***}	1.80^{***}
Error	1890	6.62	4.91	21.99	15.15	7.81	7.10

Year and temperature as fixed effects, lines as random, nested in year. df, Degrees of freedom.
Level of significance: ns, nonsignificant; $^{***}P < 0.001$.

the variability among individual flies, accounts on average for 10.6% of the total variation. Table 2 shows that the results for wing length and W/T ratio are quite similar, while those for thorax length are slightly different. For the thorax, the temperature effect is less pronounced, while the individual variability is more important.

The average reaction norms of wing and thorax length (figure 1) reveal a strong similarity among the three year samples. For both traits, the average response curves have a concave (bowed downward) shape, which will be further analysed in the following sections. The significant year × temperature interaction means that the reaction norms are not parallel. As seen in figure 1, this effect is mainly due to abnormally low values at 12 and 21°C in the 1999 sample. The W/T ratio, on the other hand, exhibits a monotonically decreasing curve with increasing temperature (see table 1 and figure 2).

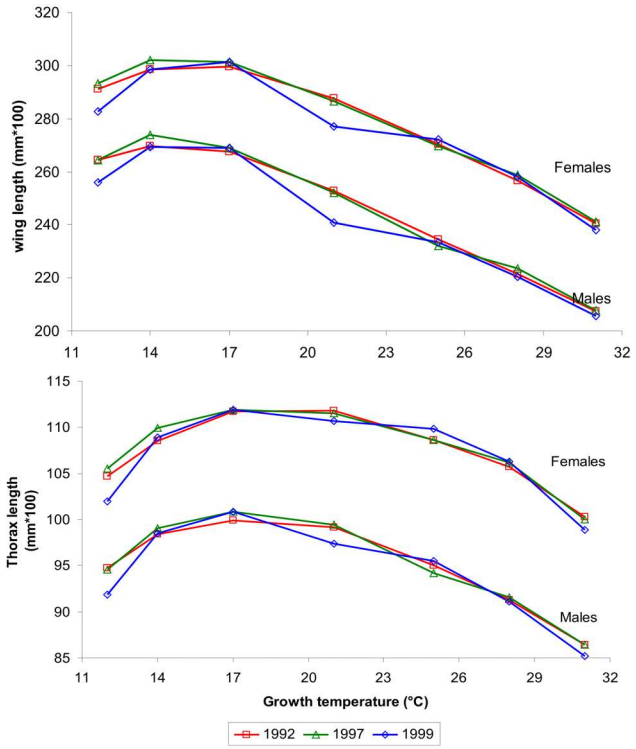

Figure 1. Average reaction norms of wing and thorax length obtained in the different year samples.

A practical conclusion of the overall similarity between the results of the different years is that, in many cases, and for the sake of simplicity, it will be possible to pool the 30 lines into a single sample, as already done in table 1.

Reaction norms of isofemale lines: The variability among isofemale lines accounts for, on average, 4% of the total variability (table 2). This variability has mainly a genetic basis and is illustrated in figure 2 for female thorax length and W/T ratio. Results for wing length are shown in another paper (David *et al*. 2005).

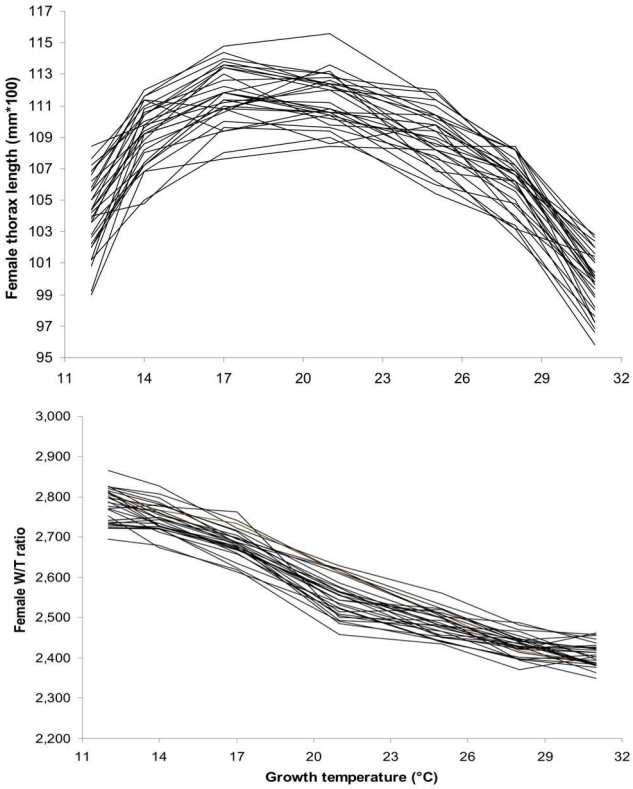

Figure 2. Reaction norms of thorax length and W/T ratio in females of the 30 isofemale lines. Notice the concave (bowed downward) shape of thorax length and the monotonically decreasing shape for the W/T ratio.

The graphs illustrate a good homogeneity in the shape of the reaction norms, and a fairly low variation among lines. For thorax length, all lines exhibit a maximum, generally between 17 and 21°C, and a decrease on both sides. The monotonically decreasing W/T ratio is visible in all lines.

The shape of reaction norms—derivative analysis: Figures 1 and 2 show that, for wing and thorax length, the reaction norms are not linear but bowed downwards. For the W/T ratio, on the other hand, the departure from linearity is less evident. So we asked two related questions: What is the precise shape of each curve? What is the best way to describe them, in biological terms?

A classical approach is to consider the shape of the derivative curves: a linear reaction norm will have a constant derivative (its slope); a quadratic reaction norm will have a linear derivative. In the present case, we could calculate an empirical derivative for each trait and line. For each interval between two successive temperatures, we calculated the difference between the two trait values, and then scaled the difference to one °C. With seven growth temperatures, we have six intervals, corresponding to mean temperatures of 13, 15.5, 19, 23, 26.5 and 29.5°C.

The average derivative curves of the three traits in females and males are shown in figure 3. ANOVAs, applied to these data (not shown), revealed in each case a highly significant effect of temperature. For wing and thorax length, there were also significant effects of year and sex, while these effects were not significant for the W/T ratio.

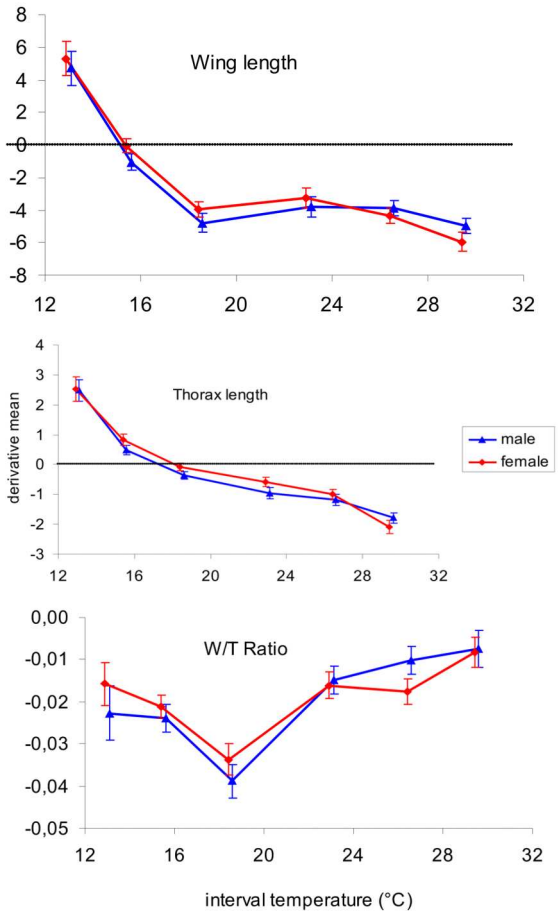

Figure 3. Average derivative curves for wing and thorax length and W/T ratio in both sexes. Each value is the mean of 30 lines and the confidence interval (2 × s.e.) is shown in each case. For wing and thorax length, the temperature for which the derivative is zero is a means for calculating the position of the maximum value.

In all cases, the year × temperature interaction was always significant, suggesting that the shapes of the derivatives were slightly different between years. However, as shown before, the differences among years were small, so that the pooled sample of 30 lines was used in further studies.

Results shown in figure 3 were subjected to a multiple regression analysis and, in each case, a simple linear model was rejected. There are, however, clear differences between traits.

For thorax length, the derivatives of male and female data are monotonically decreasing curves and a linear regression is convenient between 15.5 and 29.5°C, while the values for 13°C are slightly too high. Altogether, the departure from a straight line is not very important, so that a quadratic adjustment of the norms may be an acceptable simplification.

For wing length, the derivative curves appear biphasic: a linear decrease at low temperatures, between 13 and 19°C; then an almost horizontal line with an average value close to −4, which means that wing length decreases by 0.04 mm when growth temperature increases by 1°C. These results suggest two possible strategies for analysing the reaction norms of wing length. One is to consider a biphasic reaction norm with two parts, below and above 19°C. The other is to assume that the derivatives are not very different from a quadratic convex curve. In that case, the reaction norm itself should be adjusted to a cubic polynomial.

Finally, for the W/T ratio, the derivatives are always negative with a minimum around 19°C and maxima at extreme, low or high, temperatures. This shape can be adjusted to a quadratic convex model, so that the integral curve (the reaction norm) has a sigmoid shape and should be described by a cubic polynomial. However, as seen in figure 2, the sigmoid shape is not strongly pronounced, so that a simple, linear model might also be implemented.

The shape of reaction norms—polynomial adjustments and characteristic values: The analysis of derivative curves has revealed for each trait a fairly complex and sometimes a biphasic shape. Polynomial models are convenient for adjusting a response curve, but the major problem is to choose the polynomial degree (David *et al*. 1997, 2004). A higher degree will always provide a better fit between the observations and the model. High-degree polynomials are, however, difficult to interpret in biologically relevant terms. There is, thus, a practical tradeoff between the need to increase the polynomial degree for a better adjustment and the use of a simple polynomial for an easier biological interpretation.

The linear model is well known and most utilized in biology, with only two coefficients (intercept and slope). For a quadratic polynomial, the formula becomes:

$$P = g_0 + g_1 t + g_2 t^2, \qquad \text{(Eqn 1)}$$

where P is the phenotype, g_0, g_1 and g_2 the polynomial coefficients, and t temperature (the environmental gradient). These coefficients can be calculated, with a standard error, by the multiple-regression procedure (StatSoft 1999). Their significance is however not straightforward and successive coefficients are strongly and negatively correlated. Eqn 1 can be rewritten as (David *et al*. 1997):

$$P = \mathrm{MP} + g_2(t - \mathrm{TMV})^2, \qquad \text{(Eqn 2)}$$

where MP is the maximum (or minimum) phenotype, TMV the temperature of maximum (or minimum) value, and g_2 the curvature parameter. MP and TMV (as well as g_2) can be estimated with their standard error, using the nonlinear estimation procedure (StatSoft 1999).

These three values completely define a quadratic polynomial: they have an obvious biological significance and may be called the characteristic values of the reaction norm (David *et al*. 1997). In the sections below, we illustrate these analyses by presenting the results of quadratic adjustments for thorax and wing length, and of cubic adjustments for wing length and W/T ratio. In the latter case, cubic adjustments provided poor results, so the linear model was also used.

Adjustment of thorax length to a quadratic polynomial: For analysing the results of the 30 isofemale lines shown in figure 2, three different strategies can be implemented. Firstly, as in several previous papers (e.g. Karan *et al*. 1999) we can consider each line, and calculate its polynomial coefficients and its three characteristic values. This strategy, when possible, is certainly the best since it provides some insight about genetic variability among lines and permits further analyses and comparisons (e.g. ANOVA between years). It turns out that, sometimes, a convenient adjustment is not possible for all lines; for example, an estimated maximum value falls outside the normal thermal range (Moreteau *et al*. 2003; Gibert *et al*. 2004b). In such a case, the abnormal lines can be excluded. But it is also possible to consider only a single reaction norm for the whole data set. In this case, two possible techniques can be implemented with different statistical procedures. One possibility is to consider the matrix of the whole data set (210 values for 30 lines at seven temperatures) and calculate the polynomial coefficients and characteristic values. The other possibility is to calculate the mean value for each temperature and then make the polynomial adjustment on seven values only. The results of these three techniques are presented and compared in table 3.

In all cases, a separate analysis of each line provided coherent results, and permitted a comparison of samples from different years. For several parameters, and mainly for females, a significant heterogeneity between years was revealed by ANOVA. With the exception of g_0 in females, the magnitude of the year effect was, however, small, allowing a global analysis of the whole sample. The coefficients of variation (CVs) among lines were quite large for the three polynomial coefficients, always greater than 10 and with an average value of 16.15 ± 1.53 ($n = 6$). Much lower values were obtained for the coordinates of the maximum (MV and TMV) with practically no difference among years. Finally, the high value of R^2 (0.93 in both sexes) indicates a good fit to a quadratic reaction norm.

With the two other methods, we could compare only the mean values. A general and satisfying conclusion is that the three different techniques provided almost identical means for polynomial coefficients and characteristic values. There is however an exception concerning R^2, which is much lower, when the calculations are made on the whole matrix.

Reaction norms of wing length: As for the thorax, polynomial adjustments gave plausible results for each isofemale line. For the sake of simplicity, we present here only the mean calculations done separately on each line (table 4). There is, however, a problem concerning the degree of the polynomial adjustment to be chosen. Considering the shape of the

Table 3. Results of quadratic polynomial adjustments on reaction norms of thorax length. Results of three analytical procedures are shown. First, calculations were made separately for each isofemale line, and mean values are given for each year sample. Samples are compared by ANOVA, and the CVs (among lines) are also given. A second method is to consider the whole data set, and two possibilities are shown. One is to consider the whole matrix of isofemale lines data, corresponding to 210 mean values (30 lines × 7 temperatures). The other is to consider only the seven values of the overall mean curve. (g_0, g_1, g_2 are the polynomial coefficients; MV (maximum value) and TMV (temperature of maximal value) are the coordinates of the maximum. The goodness of fit is appreciated by R^2.)

		Isofemale lines analysis						Total set	
		1992 ($n = 10$)	1997 ($n = 10$)	1999 ($n = 10$)	Mean ($n = 30$)	CV	ANOVA	Whole matrix ($n = 210$)	Overall mean curve ($n = 7$)
g_0	♀	71.36 ± 1.87	75.08 ± 2.39	61.12 ± 2.22	69.19 ± 1.63	12.9	***	69.29 ± 1.76	69.65 ± 4.37
	♂	68.54 ± 2.36	69.31 ± 2.52	60.51 ± 2.45	66.12 ± 1.55	12.84	*	66.12 ± 1.67	66.16 ± 5.97
g_1	♀	4.058 ± 0.165	3.751 ± 0.231	4.942 ± 0.229	4.250 ± 0.150	13.39	*	4.241 ± 0.177	4.203 ± 0.441
	♂	3.330 ± 0.230	3.304 ± 0.228	4.020 ± 0.238	3.551 ± 0.143	22.09	ns	3.551 ± 0.168	3.545 ± 0.601
g_2	♀	−0.100 ± 0.004	−0.095 ± 0.005	−0.120 ± 0.005	−0.105 ± 0.003	17.43	**	−0.105 ± 0.004	−0.104 ± 0.010
	♂	−0.089 ± 0.005	−0.090 ± 0.005	−0.104 ± 0.005	−0.094 ± 0.003	18.27	ns	−0.094 ± 0.004	−0.094 ± 0.014
MV	♀	112.4 ± 0.789	112.1 ± 0.610	112.0 ± 0.514	112.2 ± 0.363	1.77	ns	112.1 ± 0.224	112.00 ± 0.56
	♂	99.58 ± 0.331	99.83 ± 0.323	99.26 ± 0.482	99.56 ± 0.219	1.20	ns	99.65 ± 0.201	99.48 ± 0.73
TMV	♀	20.25 ± 0.218	19.65 ± 0.274	20.55 ± 0.112	20.15 ± 0.137	3.72	*	20.17 ± 0.108	20.16 ± 0.27
	♂	18.48 ± 0.277	18.32 ± 0.269	19.14 ± 0.273	18.65 ± 0.166	4.81	ns	18.79 ± 0.146	18.79 ± 0.53
R^2	♀	0.946 ± 0.013	0.940 ± 0.007	0.908 ± 0.012	0.931 ± 0.007	4.69	*	0.794	0.970
	♂	0.963 ± 0.007	0.923 ± 0.007	0.907 ± 0.018	0.931 ± 0.009	5.50	*	0.852	0.959

ns, Nonsignificant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

derivative curves (figure 2), we implemented three kinds of calculation: (i) a quadratic adjustment using the data of all temperatures; (ii) a quadratic adjustment using only the low temperatures (12 to 21°C), which correspond to the linear decreasing part of the derivative; (iii) a cubic adjustment using all temperatures. Calculations are given in table 4 and illustrated in figure 4.

A general observation is that, for the polynomial coefficients, the three methods sometimes produced very different results. This can be seen by considering the variability between adjustments, expressed as a CV (last column in table 4) which has an average value of 63.9 ± 5.26 (n = 6). For example g_0, which indicates the value of the trait at 0°C, would be close to 2.5 mm with the first method, but close to 1.0 with the two others. Part of this effect arises from differences among g_2 (curvature coefficient). A stronger curvature (proportional to the absolute value of g_2) decreases the value of g_0. It is interesting to note that the first method (quadratic, all temperatures), which assumes an overall linear decrease of the derivative (figure 2), gives the lowest curvature, while the cubic adjustment provides the strongest curvature.

The variability of g_0, g_1, g_2 among lines is significantly less in the first adjustment (all temperatures) than in the second one (low temperatures only) with average CVs of 22.5 ± 5.8 and 47.7 ± 6.1 respectively (n = 6 in each case). The high variability among polynomial coefficients, when only low-temperature data are used, is explained, at least in part, by a heterogeneity among year samples (ANOVA not shown) and can be seen in figure 1. The heterogeneity, however, practically disappears when all temperatures are taken into account. With the cubic adjustment, the variability of polynomial coefficients among lines is also very high (average CV = 41.2 ± 2.5, n = 8).

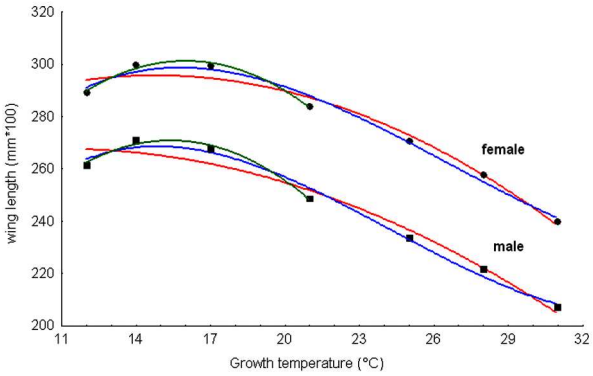

Figure 4. Results obtained with three methods of polynomial adjustment of wing length reaction norms. The filled circles and squares are the experimental points; red curves, quadratic adjustment on all temperatures; green curves, quadratic adjustment for temperatures 12 to 21°C; blue curves, cubic adjustment on all temperatures.

It is also interesting to note that the heterogeneity between lines and between adjustments strongly decreases when MV and TMV are calculated. Average CVs between lines are 4.39 ± 1.62 and 11.86 ± 4.71 for MV and TMV

Table 4. Analysis of wing length reaction norms using three kinds of polynomial adjustments: a quadratic adjustment using all temperatures, a quadratic adjustment using only the four low temperatures, a cubic adjustment using all temperatures. Each value is the mean (± s.e.) for the pool of 30 isofemale lines, CV is the coefficient of variation among lines. (R^2, coefficient of determination; g_0–g_3, polynomial coefficients; MV, maximal value; TMV, temperature of MV.)

		Quadratic (all temperatures)		Quadratic (12 to 21°C)		Cubic (all temperatures)		
		m + s.e.	CV	m + s.e.	CV	m + s.e.	CV	Variability between adjustments (CV)
g_0	♀	247.9 ± 3.802	8.40	119.2 ± 12.73	58.5	125.18 ± 11.07	48.4	44.5
	♂	248.4 ± 3.876	8.55	99.87 ± 13.38	73.4	95.41 ± 8.734	50.1	62.0
g_1	♀	6.457 ± 0.323	27.4	22.73 ± 1.558	37.5	25.74 ± 1.730	36.8	56.1
	♂	3.641 ± 0.301	45.3	22.16 ± 1.674	41.4	28.67 ± 1.754	33.4	71.5
g_3	♀	−0.218 ± 0.009	17.5	−0.710 ± 0.047	36.4	−1.160 ± 0.084	39.5	67.7
	♂	−0.157 ± 0.008	28.1	−0.719 ± 0.051	39.1	−1.389 ± 0.085	33.7	81.7
g_3	♀	–	–	–	–	0.0146 ± 0.0013	49.1	–
	♂	–	–	–	–	0.0190 ± 0.0013	38.2	–
MV	♀	296.3 ± 1.2	2.22	301.4 ± 1.117	2.03	300.14 ± 1.647	3.00	0.92
	♂	270.7 ± 2.3	4.75	271.0 ± 1.061	2.14	279.17 ± 6.228	12.2	1.76
TMV	♀	14.52 ± 0.36	13.4	15.94 ± 0.107	3.67	15.68 ± 0.190	6.62	4.15
	♂	11.25 ± 0.69	33.7	15.30 ± 0.106	3.81	14.86 ± 0.216	7.95	16.1
R^2	♀	0.955 ± 0.006	3.69	0.951 ± 0.011	6.14	0.972 ± 0.006	3.15	1.16
	♂	0.952 ± 0.006	3.42	0.960 ± 0.008	4.78	0.976 ± 0.004	2.45	1.27

respectively (n = 6 in each case). Also, the variability among the three methods is small, except in the case of male TMV.

Finally, R^2 values were all very high, in all cases greater than 0.95, and the three methods all apparently provided an excellent fit between calculated and observed values. In other words, considering R^2 is of little help for choosing a method and the main question remains 'What is the best adjustment for the reaction norm of wing length?'.

The comparison of characteristic values in table 4 reveals a major discrepancy between the methods, which is the low value (11.25°C) of male TMV after a quadratic adjustment made on all temperatures, accompanied by a big heterogeneity among lines (CV of 33.7). In fact, six lines could be considered as abnormal, that is providing nonplausible TMVs, either below 7°C or above 24°C.

We eliminated these six lines, but the average TMV was not much changed (11.9°C) although the CV was reduced almost by half (15.9). We also analysed the whole data matrix with this method, but the TMV remained very low (11.03°C).

Looking at the experimental data (figures. 1, 3, 4) we consider that the true average TMV for male wing is close to 15°C. In other words, a quadratic polynomial is not satisfactory. A cubic polynomial should be preferred since plausible estimates of MV and TMV were obtained in all lines and for both sexes. A cubic adjustment provides convenient values of the coordinates of the maximum. It is however not possible to estimate an overall curvature, since g_2 of a cubic polynomial is not equivalent to g_2 of a quadratic one.

Reaction norms of W/T ratio: Because of the curvilinear shape of the derivative (figure 2), which suggests a sigmoid decreasing norm for the ratio, we first used a cubic polynomial adjustment. This procedure, however, when applied to each isofemale line, produced numerous (seven for females, 10 for males) inconsistent results, such as a temperature of inflection point exceeding 50°C. We think these aberrant lines were too numerous for being excluded from the calculations. So we provide (table 5) only the characteristic values calculated either on the whole data set or on the overall mean curve.

The results of the two methods are coherent. The inflection point is around 19°C in females and 17°C in males, as illustrated in figure 5. The overall norm of males is slightly shifted to the left with respect to that of females, but the two curves are very close.

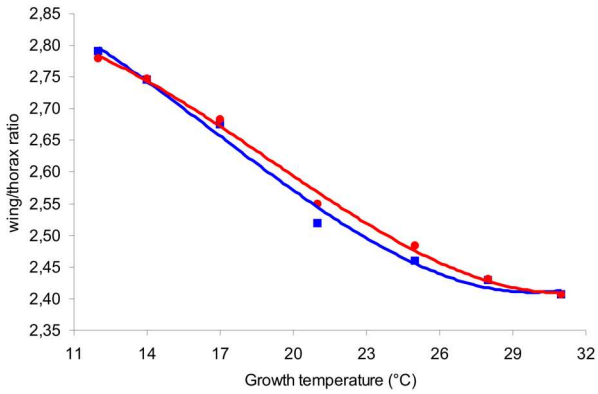

Figure 5. Average reaction norms (cubic adjustment) of W/T ratio in males (■) and females (●).

The goodness of fit to the cubic polynomial may be appreciated by the coefficient of determination, R^2. It is interesting to mention that all individual adjustments provided very high R^2 (average over 0.98) and that aberrant lines also gave very high values. Again a high R^2 is not an indication of a convenient adjustment. Since the reaction norms of all lines were monotonically decreasing and not very different from a straight line, we also made linear adjustments (table 6) for the individual lines and for the total data set (two methods). In all cases, R^2 values greater than 0.90 were obtained, and the mean values provided by the three methods were very similar.

Table 5. Analysis of the reaction norms of W/T ratio with a cubic adjustment, using two different methods applied to the whole data set. (PIP, Phenotype at inflection point; TIP, temperature of inflection point; maxP, maximum phenotype; minP, minimum phenotype; TmaxP, maximum temperature; TminP, minimum temperature; R^2, coefficient of determination.)

	Female		Male	
Trait	Whole matrix (n = 210)	Average curve (n = 7)	Whole matrix (n = 210)	Average curve (n = 7)
PIP	2.616	2.616	2.667	2.662
TIP (°C)	19.10	19.09	16.68	16.78
Slope at IP	−0.026	−0.027	−0.029	−0.029
maxP	2.829	2.831	2.917	2.918
minP	2.404	2.401	2.418	2.407
TmaxP (°C)	7.07	6.94	3.77	3.68
TminP (°C)	31.1	31.2	29.6	29.9
R^2	0.939	0.996	0.938	0.992

Considering the individual lines, we see that the slope was much more variable than the intercept. The relationship between slope and intercept (figure 6) reveals a strong negative correlation between the two parameters, which is a general property of polynomial adjustments. From a biological point of view, and for comparing the lines, it is better to consider the mean phenotype not at 0°C, but at the average experimental temperature of 21°C. In that case, the negative correlation with slope practically disappears (figure 6).

The logarithmic transformation: Biometrical data are often transformed into logarithmic values (Sokal and Rohlf 1995). This procedure has the advantage of providing more correct statistical analyses, especially when the variance and the mean are correlated. Here we asked the question: how does this transformation modify the shape of a reaction norm and its characteristic values?

As an example, we present (table 7) the results obtained for the characteristic values of wing and thorax length after quadratic or cubic adjustments. As expected, the maximum values are completely different, owing to the log transformation, and the same conclusion applies to the polynomial coefficients (not shown). However, the temperatures of maximum values are very close to those calculated with the non-transformed data (see tables 3 and 4). In other words, if we consider Eqn 2, the log transformation changes MV and g_2, but not TMV, which is the position of the maximum along the temperature axis, and a most interesting bit of information on phenotypic plasticity.

Analysis of variability parameters

With an isofemale line design, the total phenotypic variance can be split into two components: the within-line variance and the between-line variance (David *et al*. 2005). The former mainly reflects environmental effects, while the latter corresponds mostly to a genetic component (David *et al*. 2005). It is interesting to consider the variance itself and to see how it is influenced by the environment, as done for example by Noach *et al*. (1996). It is, however, more convenient to consider a relative measure, the coefficient of variation (CV), which allows a comparison of traits with different mean values.

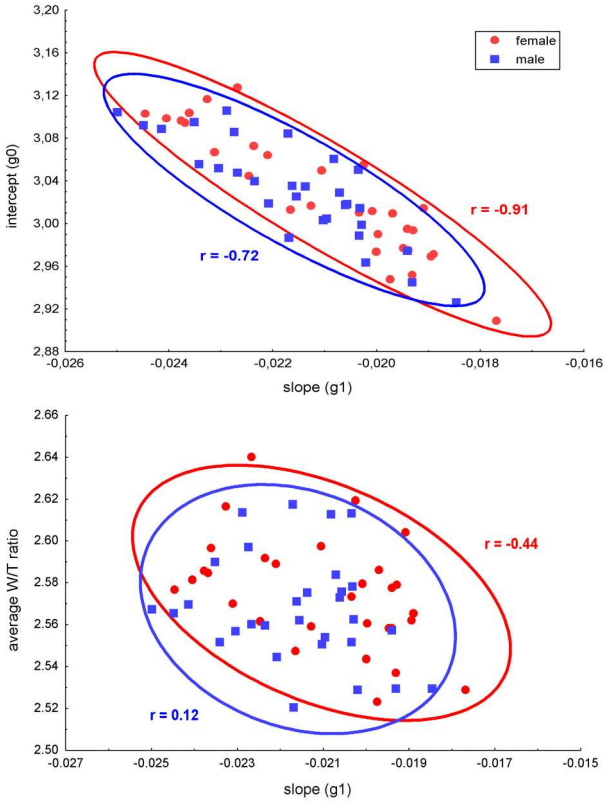

Figure 6. Results of a linear adjustment ($y = g_0 + g_1 t$) on W/T ratio in females (●) and males (■). Each value is the mean of an isofemale line. For each sex, ellipses of 95% confidence are drawn. Upper graph: relationship between slope (g_1) and intercept (g_0); the two coefficients are negatively correlated. Lower graph: relationship between slope and average value of W/T ratio (the value calculated for a temperature of 21°C); the correlations become non-significant).

Table 6. Results of a linear adjustment on the W/T ratio. Comparison of three methods is shown: individual adjustment on each isofemale line, and general adjustment on the whole matrix (210 values) or on the average curve (7 values). Mean phenotype is the W/T ratio for the mean temperature of the environment (21°C); CV is the coefficient of variation among lines.

		Individual lines (n = 30)		Whole matrix	Average curve
		m + s.e.	CV	m + s.e.	m + s.e.
Intercept (g_0)	♀	3.027 ± 0.011	1.90	3.027 ± 0.009	3.028 ± 0.031
	♂	3.031 ± 0.009	1.55	3.031 ± 0.011	3.031 ± 0.049
Slope (g_1)	♀	−0.0210 ± 0.0004	9.04	−0.0210 ± 0.0004	−0.021 ± 0.0014
	♂	−0.0216 ± 0.0003	7.34	−0.0216 ± 0.0005	−0.022 ± 0.0021
Mean phenotype	♀	2.575 ± 0.005	1.02	2.583 ± 0.003	2.583 ± 0.058
	♂	2.567 ± 0.005	1.00	2.575 ± 0.003	2.575 ± 0.060
R^2	♀	0.961 ± 0.006	3.20	0.923	0.978
	♂	0.932 ± 0.009	5.04	0.900	0.952

Table 7. Results of a logarithmic transformation of wing and thorax length characteristic values (MV, maximum value; TMV, temperature of maximum value are given, as well as R^2).

		MV	TMV	R^2
Wing (quadratic)	♀	2.471	15.11	0.901
	♂	2.426	11.98	0.921
Thorax (quadratic)	♀	2.050	20.16	0.796
	♂	1.998	18.79	0.860
Wing (cubic)	♀	2.474	15.70	0.910
	♂	2.427	14.72	0.935

The within-line variability: We first considered the within-line variance, and the results obtained for wing and thorax length of the 30 lines are illustrated in figure 7. A statistical analysis revealed for both traits significant variations according to temperature. Looking at the graph, we see that greater values are observed at extreme temperature, and a minimum around 21°C, which is easier to detect after a quadratic polynomial adjustment.

A major feature of figure 7 is that the variance of thorax length is more than five times less than the variance of wing length. This is due to a scaling effect, that is the smaller size of thorax compared to the wing. To get rid of this scaling effect, we use the CV. The results are presented in figure 8 for wing and thorax length, and also for the W/T ratio. In each case, an almost symmetrical increase of the variability is observed at extreme temperatures, with a minimum at intermediate temperature. For each trait, the data can be adjusted to a quadratic polynomial (see figure 8) which describes the reaction norm of the within-line CV. Two interesting observations can be made. Firstly, the difference between wing and thorax disappears: both traits have the same relative variability. Secondly, the variability of the W/T ratio is less than that of the traits. This is a classic observation explained by the positive correlation between the two traits.

We tried to get a better analysis of the reaction norms of the CVs. At the level of each line, CVs were, as expected, so variable that we could not, in most cases, obtain a convenient quadratic adjustment. Such an adjustment was however possible when the 10 lines corresponding to each year of collection were pooled into a single sample. The parameters of the reaction norms for the CVs of the three traits are given in table 8. ANOVA (not shown), applied to these data, failed to show any significant difference due to either year, sex or trait, for TminV (temperature of minimum variation in this case) or g_2. The overall average values are TMV = 21.25 ± 0.23°C and g_2 = 0.0077 ± 0.0010 (n = 6 in each case). There was however a significant difference for TminV between W/T ratio (average 1.34 ± 0.11) and the two size traits (average 1.80 ± 0.05).

The between-line, genetic variability: With a full-sib design, we can calculate a genetic variance which is slightly less than the between-line variance, calculated directly from the family means (see David *et al*. 2005). For each sex and temperature, we have 30 observations only, and we calculated the variance from these data. It turned out that results between sexes were quite variable and that the best way to increase

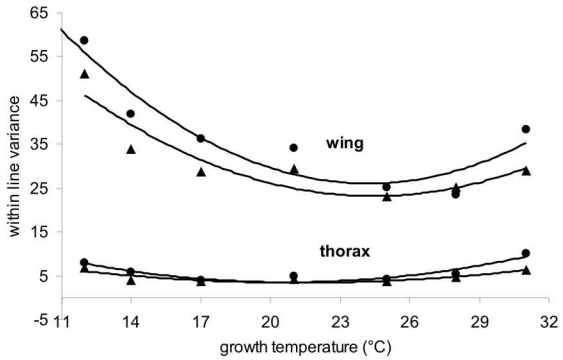

Figure 7. Variation of the within-line variance for wing and thorax length in females (•) and males (▲). Each point is based on 270 degrees of freedom. Data are adjusted to quadratic polynomials.

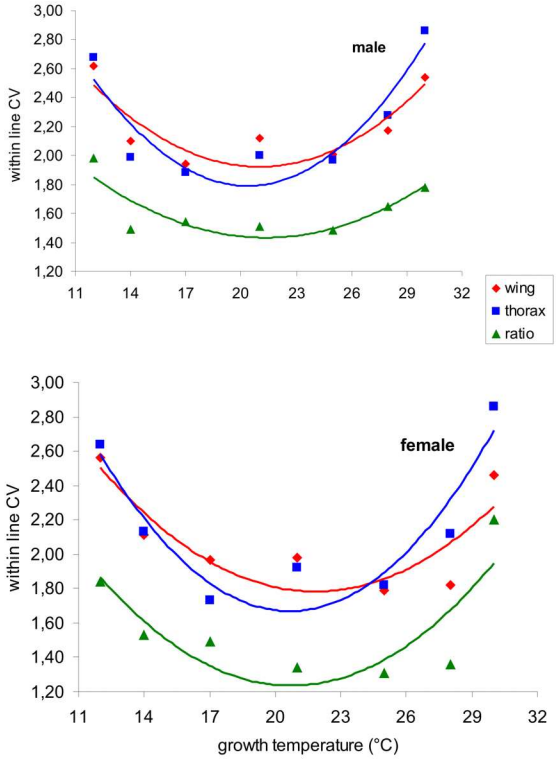

Figure 8. Variation of the within-line CV according to growth temperature in the three traits investigated. In each case, a reaction norm is drawn from a quadratic adjustment.

the precision of the analysis was to pool the female and male data. As previously, the variance was scaled to the mean, and the variations of the genetic CV, also called evolvability (Houle 1992), are shown in figure 9 (upper part).

Table 8. Analysis of the reaction norms of the within-line CV, after a quadratic adjustment. Characteristic values are given for each year, and also the R^2 parameter. (MinV, minimum value; TminV: temperature of minimum value; g_2, curvature; mean is the mean of the three years; s.e., standard error.)

		Wing length				Thorax length				W/T ratio			
		MinV	TminV	g_2	R^2	MinV	TminV	g_2	R^2	MinV	TminV	g_2	R^2
1992	♀	1.752	24.39	0.0034	0.891	1.574	21.69	0.0068	0.732	1.195	23.64	0.0044	0.944
	♂	1.718	22.36	0.0063	0.775	1.781	21.09	0.0060	0.594	1.181	22.05	0.0043	0.756
1997	♀	1.815	21.16	0.0072	0.593	1.843	20.81	0.0084	0.733	1.237	20.86	0.089	0.656
	♂	2.060	19.85	0.0059	0.697	1.784	20.44	0.0110	0.950	1.483	20.92	0.0045	0.627
1999	♀	1.747	22.00	0.0106	0.796	1.638	20.70	0.0177	0.966	1.221	19.96	0.0103	0.697
	♂	1.992	21.39	0.0068	0.659	1.882	20.40	0.0113	0.780	1.674	22.18	0.0038	0.364
mean	♀	1.771	22.51	0.0070	0.760	1.685	21.07	0.0110	0.810	1.218	21.49	0.0079	0.766
	s.e.	0.028	0.353	0.0429	0.175	0.108	0.118	0.0563	0.190	0.0194	0.413	0.0348	0.178
	♂	1.923	21.20	0.0064	0.710	1.816	20.64	0.0094	0.775	1.446	21.72	0.0042	0.582
	s.e.	0.131	0.275	0.0053	0.070	0.043	0.085	0.0304	0.202	0.2070	0.149	0.0059	0.262

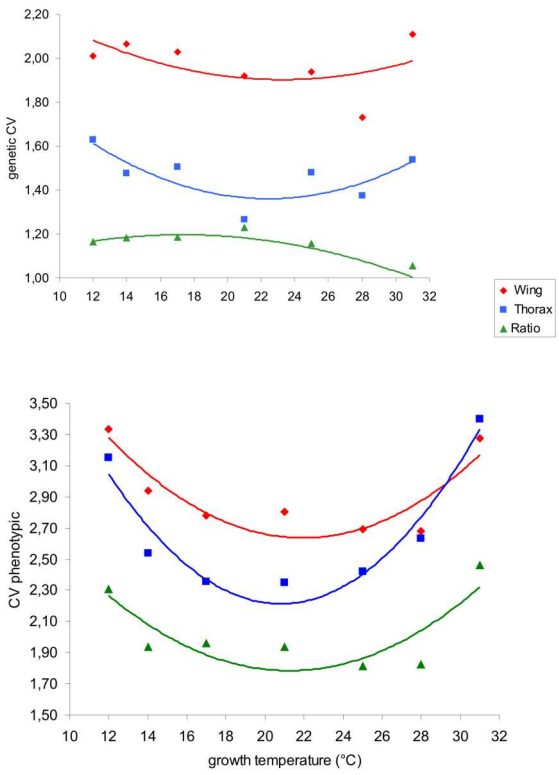

Figure 9. Upper: Variation of the genetic CV (pooled data of both sexes) according to growth temperature; notice the major difference between wing and thorax; quadratic adjustments (either concave or convex) are shown, but the curvatures are not significant. Lower: Reaction norms of the total phenotypic CV for the three traits investigated; significant quadratic adjustments are shown.

Curvilinear adjustments were made for the three traits. Both wing and thorax length gave concave reaction norms, while a convex shape was observed for the W/T ratio. However, a multiple-regression analysis revealed that, for each trait, the curvature parameter was never significantly different from zero. In other words, each reaction norm could be drawn as a horizontal line. There is a major difference between the traits. The average genetic CV is much less for the thorax (1.47 ± 0.044, n = 7) than for the wing (1.97 ± 0.048, n = 7) and still lower for the W/T ratio (1.14 ± 0.033) (n = 7 in each case).

We also calculated the intraclass correlation coefficient (ICC), which is akin to the heritability (David *et al*. 2005), and results are given in table 9. ANOVA (not shown) failed to reveal any sex or temperature effect. There is however a significant difference among traits: the ICC is higher for the wing (0.45) than for the thorax (0.31) or the W/T ratio (0.33).

Finally, we also considered the total phenotypic variability, still by calculating a CV, and the reaction norms are presented in figure 9 (lower). For each trait, significantly convex reaction norms are observed. The overall shape is most influenced by the within-line variability, which has 270 degrees of freedom against 29 for the between-line variability. The mean values are higher than those found either for the within-line CV or the between-line CV, owing to an overall increased variability. The calculated minimum values are 2.93 ± 0.10, 2.69 ± 0.16 and 2.04 ± 0.094 for wing length, thorax length and W/T ratio respectively (n = 7).

Discussion

Methodology

With modern techniques, it is possible to make numerous calculations on a data set which by itself is not huge (2100 flies of each sex). In this paper, we have tried to illustrate various analytical possibilities, without however presenting exhaustive results. Here we summarize the main conclusions.

A reaction norm is the variation of a trait along an environmental gradient, in the present case temperature. As seen below, the reaction-norm approach may be applied not only

Table 9. Coefficient of intraclass correlation (ICC), calculated on the whole data set of 30 lines at each experimental temperature.

		12°C	14°C	17°C	21°C	25°C	28°C	31°C	Mean + s.e.
Wing length	♀	0.38	0.48	0.47	0.44	0.54	0.48	0.41	0.46 ± 0.02
	♂	0.33	0.47	0.54	0.46	0.46	0.35	0.40	0.43 ± 0.03
Thorax length	♀	0.26	0.34	0.42	0.31	0.39	0.31	0.24	0.32 ± 0.03
	♂	0.26	0.31	0.36	0.26	0.35	0.26	0.23	0.29 ± 0.02
W/T ratio	♀	0.33	0.32	0.35	0.43	0.44	0.34	0.17	0.34 ± 0.03
	♂	0.21	0.39	0.40	0.37	0.26	0.25	0.34	0.32 ± 0.03

to mean values but also to the variance parameters.

For choosing a mathematical model, the first step is to consider the overall shape of the average curve. Polynomial adjustments, of various degrees, seem to be a general, all-purpose method (David *et al*. 1997) although other possibilities may be considered, for example a logistic curve (Gibert *et al*. 1998b). It is well known that the precision of an adjustment increases with the degree of the polynomial, while the biological significance tends to disappear among too many coefficients. Practically, only first-order, second-order and third-order polynomials seem to be biologically convenient. The validity of an adjustment may be appreciated by R^2, but this may be misleading: it is often possible to obtain a high R^2 for a very implausible result such as a temperature of maximum value above 100°C. Attention must always be paid to the plausibility of the results. For determining the exact shape of a reaction norm, the best procedure is to analyse the shape of the derivative curve, when this is possible (for example with isofemale lines). We did this for the three traits investigated, but with mixed results. For thorax length, we obtained a monotonically decreasing curve, which however was somehow bent and not linear. The departure from linearity was however not too much, and we could use, for this trait, a quadratic polynomial adjustment which implies a linearly decreasing derivative. For wing length, the shape of the derivative was unusual, and clearly biphasic: a rapid linear decrease at low temperatures, then an almost horizontal line above 19°C. We tried to make a biphasic adjustment, but the results of a quadratic polynomial between 12 and 21°C gave some implausible data (figure 4 and table 4). So the best conclusion is that, for calculating characteristic values and estimating the coordinates of the maximum, a cubic polynomial adjustment is preferable. For the W/T ratio, we clearly obtained a quadratic derivative (figure 3) with a minimum (the inflection point) at an intermediate temperature. This shows that the trait follows a decreasing sigmoid norm (figure 5). We then tried to adjust each line to a cubic polynomial but several lines produced implausible results, leading us to reject this model. Moreover, as seen in figure 5, the overall shape of the norms is not far from linearity, so that a linear regression proved to be a convenient approximation.

So, for the three traits investigated, we suggest a linear adjustment for the W/T ratio, a quadratic adjustment for thorax length, and a cubic adjustment for wing length. These adjustments permit calculation of characteristic values, which have a clear biological meaning. The reaction norm of W/T ratio is characterized by the slope and the mean value. The reaction norm of thorax length is characterized by the coordinates of the maximum (MV and TMV), and also by a curvature parameter. For the cubic adjustment of wing length, we also calculate the coordinates of a maximum, but a curvature parameter is not available.

Finally, we compared the real data with their log transformation. As expected, mean values were completely modified, but the TMVs did not exhibit a major change. A practical conclusion is that logs should not be used when analysing the shapes of reaction norms.

Reaction norms of trait values

Both wing and thorax lengths are related to body size, and are often considered as substitutes, for example when investigating latitudinal clines (Capy *et al*. 1993; Gibert *et al*. 2004a). Our data, however, demonstrate clearly that the two traits are not equivalent. Not only are the shapes of the norms (quadratic or cubic) different, but the TMVs also are different, around 15°C for the wing and 19.5°C for the thorax. The genetic basis of this difference is not known but it seems to be a quite general property among *Drosophila* species (David *et al*. 1997, 2004; Azevedo *et al*. 1998; Karan *et al*. 1999; Morin *et al*. 1999).

In many investigations, wing length, wing area or wing shape is considered as an estimate of size, and numerous latitudinal clines are based on wing variation (Azevedo *et al*. 1998; Karan *et al*. 1999; Gilchrist *et al*. 2000; Huey *et al*. 2000). As seen from our data, wing length is a monotonically decreasing function of temperature from 15°C up to 31°C. As a simplification, it is often said that a parallelism exists between phenotypic plasticity and clines: in both cases, smaller flies are observed at warmer temperatures (Atkinson and Sibly 1997). This parallelism is often taken as an adaptive argument, demonstrating that it is better to be smaller in warmer environments, even if the precise mechanisms remain to be identified (Pétavy *et al*. 1997; Azevedo *et al*. 1998; Bochdanovits and de Jong 2003a,b; Blanckenhorn and Demont 2004). In most allometric investigations, body weight is considered as the best estimate of body size (Peters 1983; Reiss 1989; Harvey and Pagel 1991). In *Drosophila*, however, weight is difficult to estimate and

varies according to age and feeding conditions (Capy *et al*. 1993; Karan *et al*. 1999). Weight seems however more akin to thorax size than to wing size (Barker and Krebs 1995; Karan *et al*. 1998). We thus suggest that thorax length is a better estimate of body size than wing length. Under this assumption, we cannot say that body size decreases regularly with temperature, and hence the temperature–size rule is invalid. Since the maximum size is observed at an intermediate temperature, close to the middle of the thermal range (21°C), a better adaptive interpretation would be that size is maximum under optimal physiological conditions and exhibits deleterious effects of colder or warmer temperatures, resulting in a cold or heat stress, and finally 100% mortality below 12°C or above 32°C (Pétavy *et al*. 2001). Such an optimum interpretation also seemed evident for ovariole number, which is directly related to fitness (offspring production) and is at a maximum at 21°C (Delpuech *et al*. 1995).

Why the maximum wing length is observed at a much lower temperature is not clear, but we also have an adaptive interpretation, first proposed by Stalker (1980) for genetical seasonal variations, and extended by Pétavy *et al*. (1997) for plasticity. The almost linear decrease of the W/T ratio with growth temperature implies a linear increase of wing loading (Pétavy *et al*. 1997). The phenotypic plasticity might be selected not directly on size, but on wing loading. Higher wing loading must be accompanied by faster wing beat, which is possible only in a warm environment (Pétavy *et al*. 1997). Under cold conditions, wing beat is possible only at a lower frequency, and flight efficiency is favoured by decrease in wing loading. In the case of *D. melanogaster*, we know the direction of evolution, from tropical Africa (ancestral populations) to temperate countries (David and Capy 1988). During the conquest of colder places, selection acted to improve flight in the cold. This was realized by increasing wing area. Owing to a positive genetic correlation between wing area and body size, this phenomenon resulted in the latitudinal size cline presently observed.

Latitudinal variations are not only seen for size traits, but also for shape of the reaction norms. It is now quite clear that, in several species and for different traits, TMVs have a higher value in tropical populations (Delpuech *et al*. 1995; Morin *et al*. 1999; Moreteau *et al*. 2003). In other words, the thermal optimum is higher when populations are adapted to warmer conditions.

Reaction norms of variability and CV

Most evolutionary investigations have thus far considered the mean values of the traits. The variance among individuals, which is notoriously less precise than the mean (Sokal and Rohlf 1995), has remained relatively neglected, except for calculating parameters which, like heritability, may predict the response to selection (Falconer and Mackay 1996).

There is, however, a recent and renewed interest in the variance, either in its nonheritable environmental component (Debat and David 2001; Polak 2003; Zhang and Hill 2005) or in its genetic component, and its capacity to change under stress conditions (Bijlsma and Loeschcke 1997; Hoffmann and Parsons 1997; Hoffmann and Hercus 2000). With an isofemale line design, we could estimate the between-line (genetic) variance, and the within-line variance, which mainly expresses an environmental component (David *et al*. 2005). Since there is a relationship between mean and variance, all measurements were scaled to the mean by calculating a CV. At the within-line level, which is more precise since it is based on more numerous observations, we found that using a CV suppressed the difference between wing and thorax length. For the W/T ratio, the CVs were clearly less, as expected for a ratio of two variables that are positively correlated. But the main result is that CVs are variable among environments and, in this respect, exhibit a specific convex reaction norm, with a minimum at an intermediate temperature. It is especially interesting to notice that the temperature of minimum variability (TminV) is the same for the two sexes and the three traits investigated (table 8), with an average value of 21.25 ± 0.23°C. Clearly, trait values and variability are disconnected and, in the case of variability, it is easy to argue that the minimum corresponds to a physiological optimum.

The analysis of genetic variability (genetic CV), based on a smaller number of observations, failed to show any significant effect of temperature. We are aware of only one publication (Noach *et al*. 1996) in which two populations of *D. melanogaster* from Europe and Africa were compared with respect to phenotypic plasticity with an isofemale line design. The authors found a strong curvature (a convex norm) for the genetic variance of wing and thorax length, with a minimum around 23–24°C, but in the African population only. Curiously, the within-line, environmental variance was not strongly affected by temperature. There is a possibility that this discrepancy is due to the geographic origin, since the European population investigated by Noach *et al*. (1996) failed to show a significant variation of its genetic variability along the temperature gradient.

Since in our study the environmental and genetic variance reacted differently to temperature, we should observe a decrease of heritability, or of the intraclass correlation at extreme temperatures (Hoffmann and Merilä 1999). We did not, however, find a significant effect, presumably owing to the fact that the observed differences remained quite small. The fact that genetic CV of thorax length is much less than that of wing length accounts for the lower heritability of this trait (David *et al*. 2005).

The relationship between environmental stress and genetic variability is a hotly debated topic. Several investigators have argued that, under stressful conditions, a cryptic genetic variability could be unravelled, leading to the possibility of a faster adaptive response (Bijlsma and Loeschcke 1997; Hoffmann and Parsons 1997; Rutherford and Lindquist 1998; Gibson and Dworkin 2004). Experimental results on genetic variability, however, seem quite variable, according

to the trait, the stress or the species investigated (Imasheva *et al*. 2000; Pétavy *et al*. 2004). A more general conclusion is the increase in the phenotypic and nongenetic variance under stressing conditions (Delpuech *et al*. 1995; Imasheva *et al*. 1997; Pétavy *et al*. 1997; Moreteau *et al*. 2003).

Sampling and analysing natural populations

Latitudinal clines for wing and thorax length have been documented in numerous populations of *D. melanogaster* (Capy *et al*. 1993). However, there was a broad variability of the populations around the regression line. There are two possible interpretations: either these variations were due to genetic drift in the laboratory, or they truly reflected significant differences among populations from similar latitudes. There is yet no definitive answer to these alternatives. Our results, concerning three independent year samples from the same locality, point however to a genetic stability in a given place. If this phenomenon was confirmed, it would be better to sample repeatedly in the same place instead of keeping a set of isofemale lines over years. We also investigated, with the same procedure of isofemale lines, two populations from the vicinity of Paris, about 500 km from Bordeaux (Pétavy *et al*. 2004). The mean trait values (at 25°C only) were very similar to those of the Bordeaux population. In other words, we suggest that, for quantitative traits, all French populations are similar and submitted to the same kind of (unknown) stabilizing selection. We are, however, aware (unpublished results) of populations living in different places but at the same latitude, close to the Equator, that exhibit significant morphological differences. Such differences suggest that phenotypes are not selected by climatic conditions only. This problem obviously deserves further investigations in specific places, using the same protocol as in Bordeaux, that is isofemale lines in their second laboratory generation. Of course, this means a long-term investigation and a large amount of data. On the other hand, we have shown that a rigorous experimental design, and use of a convenient highly nutritive rearing medium, provide stable data at least in the laboratory. We suggest that, in the future, morphometrical data should be collected in a way that would be exactly the same in different laboratories, permitting the creation of a large database, accessible to all interested investigators.

References

Angilletta M. J. and Dunham A. E. 2003 The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. *Am. Nat*. **162**, 332–342.

Angilletta M. J., Steury T. S. and Sears M. W. 2004 Temperature growth rate and body size in ectotherms: fitting pieces of a life history puzzle. *Integr. Comp. Biol*. **44**, 498–509.

Atkinson D. 1994 Temperature and organism size—a biological law for ectotherms? *Adv. Ecol. Res*. **25**, 1–58.

Atkinson D. and Sibly R. M. 1997 Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends Ecol. Evol*. **12**, 235–239.

Azevedo R. B. R., James A. C., McCabe J. and Partridge L. 1998 Latitudinal variation of wing : thorax size ratio and wing-aspect ratio in *Drosophila melanogaster. Evolution* **52**, 1353–1362.

Barker J. S. F. and Krebs R. A. 1995 Genetic variation and plasticity of thorax length and wing length in *Drosophila aldrichi* and *D. buzzatii. J. Evol. Biol*. **8**, 689–709.

Bergmann C. 1847 Uber die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Gött. Stud.* **3**, 595–708.

Bijlsma R. and Loeschcke V. 1997 *Environmental stress, adaptation and evolution*. Birkhäuser, Basel.

Blanckenhorn W. U. 2000 The evolution of body size: what keeps organisms small? *Q. Rev. Biol*. **75**, 385–407.

Blanckenhorn W. U. and Demont M. 2004 Bergmann and Converse Bergmann latitudinal clines in arthropods: two ends of a continuum? *Integr. Comp. Biol*. **44**, 413–424.

Bochdanovits Z. and de Jong G. 2003a Temperature dependence of fitness components in geographical populations of *Drosophila melanogaster*: changing the association between size and fitness. *Biol. J. Linn. Soc*. **80**, 717–725.

Bochdanovits Z. and de Jong G. 2003b Temperature dependent larval resource allocation shaping adult body size in *Drosophila melanogaster. J. Evol. Biol*. **16**, 1159–1167.

Capy P., Pla E. and David J. R. 1993 Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans*. I. Geographic variations. *Genet. Sel. Evol*. **25**, 517–536.

Cavicchi S., Guerra D., Giorgi G. and Pezzoli C. 1985 Temperature related divergence in experimental populations of *Drosophila melanogaster*. I. Genetic and developmental basis of wing size and shape variation. *Genetics* **109**, 665–689.

Coyne J. A. and Beecham E. 1987 Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster. Genetics* **117**, 727–737.

David J. R. and Capy P. 1988 Genetic variation of *Drosophila melanogaster* natural populations. *Trends Genet*. **4**, 106–111.

David J. R. and Clavel M. F. 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 J. R. and Kitagawa O. 1982 Possible similarities in ethanol tolerance and latitudinal variations between *Drosophila virilis* and *D. melanogaster. Jpn. J. Genet*. **57**, 89–95.

David J. R., Moreteau B., Gauthier J. R., Pétavy G., Stockel J. and 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.

David J. R., Gibert P., Gravot E., Pétavy G., Morin J. P., Karan D. and Moreteau B. 1997 Phenotypic plasticity and developmental temperature in *Drosophila*: analysis and significance of reaction norms of morphometrical traits. *J. Therm. Biol*. **22**, 441–451.

David J. R., Gibert P. and Moreteau B. 2004 Evolution of reaction norms. In *Phenotypic plasticity: functional and conceptual approaches* (ed. T. J. DeWitt and S. M. Scheiner), pp. 50–63. Oxford University Press, New York.

David J. R., Gibert P., Legout H., Capy P. and Moreteau B. 2005 Isofemale lines in *Drosophila*: an empirical approach to quantitative traits analysis in natural populations. *Heredity* **94**, 3–12.

Debat V. and David P. 2001 Mapping phenotypes: canalization, plasticity and developmental stability. *Trends Ecol. Evol*. **16**, 555–561.

Delpuech J. M., Moreteau B., Chiche J., Pla E., Vouidibio J. and David J. R. 1995 Phenotypic plasticity and reaction norms in temperate and tropical populations of *Drosophila melanogaster*. Ovarian size and developmental temperatures. *Evolution* **49**, 670–675.

Falconer D. S. and Mackay T. F. C. 1996 *Introduction to quantita-*

tive genetics. Longman, London.

Gibert P. and de Jong G. 2001 Temperature dependence of development rate and adult size in *Drosophila* species: biophysical parameters. *J. Therm. Biol*. **14**, 267–276.

Gibert P., Moreteau B., Moreteau J. C. and David J. R. 1998a Genetic variability of quantitative traits in *Drosophila melanogaster* (fruit fly) natural populations: analysis of wild living flies and of several laboratory generations. *Heredity* **80**, 326–335.

Gibert P., Moreteau B., David J. R. and Scheiner S. 1998b Describing the evolution of reaction norm shape: body pigmentation in *Drosophila. Evolution* **52**, 1501–1506.

Gibert P., Capy P., Imasheva A., Moreteau B., Morin J. P., Pétavy G. and David J. R. 2004a Comparative analysis of morphometrical traits among *Drosophila melanogaster* and *D. simulans*: genetic variability, clines and phenotypic plasticity. *Genetica* **120**, 165–179.

Gibert P., Moreteau B. and David J. R. 2004b Phenotypic plasticity of body pigmentation in *Drosophila melanogaster*: genetic repeatability of quantitative parameters in two successive generations. *Heredity* **92**, 499–507.

Gibson G. and Dworkin I. 2004 Uncovering cryptic genetic variation. *Nat. Rev. Genet*. **5**, 681–690.

Gilchrist A. S. and Partridge L. 1999 A comparison of the genetic basis of wing size divergence in three parallel body size clines of *Drosophila melanogaster*. *Genetics* **153**, 1775–1787.

Gilchrist A. S., Azevedo R. B., Partridge L. and O'Higgins P. 2000 Adaptation and constraint in the evolution of *Drosophila melanogaster* wing shape. *Evol. Dev.* **2**, 114–124.

Harvey P. H. and Pagel M. D. 1991 *The comparative method in evolutionary biology*. Oxford University Press, Oxford.

Hoffmann A. A. and Hercus M. J. 2000 Environmental stress as an evolutionary force. *Bioscience* **50**, 217–226.

Hoffmann A. A. and Merilä J. 1999 Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol. Evol*. **14**, 96–101.

Hoffmann A. A. and Parsons P. A. 1997 *Extreme environmental change and evolution*. Cambridge University Press, Cambridge.

Houle D. 1992 Comparing evolvability and variability of quantitative traits. *Genetics* **130**, 195–204.

Huey R. B., Gilchrist G. W., Carlson M. L., Berrigan D. and Serra L. 2000 Rapid evolution of a geographic cline in size in an introduced fly. *Science* **287**, 308–309.

Imasheva A. G., Bubli O. A. and Lazebny O. E. 1994 Variation in wing length in Eurasian natural populations of *Drosophila melanogaster*. *Heredity* **72**, 508–514.

Imasheva A. G., Loeschcke V., Zhivotovsky L. A. and Lazebny O. E. 1997 Effects of extreme temperatures on phenotypic variation and developmental stability in *Drosophila melanogaster* and *D. buzzatii*. *Biol. J. Linn. Soc*. **61**, 117–126.

Imasheva A. G., Moreteau B. and David J. R. 2000 Growth temperature and genetic variability of wing dimensions in *Drosophila*: opposite trends in two sibling species. *Genet. Res*. **76**, 237–247.

James A. C., Azevedo R. B. and Partridge L. 1997 Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics* **146**, 881–890.

Karan D., Munjal A. K., Gibert P., Moreteau B., Parkash R. and David J. R. 1998 Latitudinal clines for morphometrical traits in *Drosophila kikkawai*: a study of natural populations from the Indian subcontinent. *Genet. Res.*. **71**, 31–38.

Karan D., Morin J. P., Gravot E., Moreteau B. and David J. R. 1999 Body size reaction norms in *Drosophila melanogaster*: temporal stability and genetic architecture in a natural population. *Genet. Sel. Evol*. **31**, 491–508.

Karan D., Dubey S., Moreteau B., Parkash R. and David J. R. 2000 Geographical clines for quantitative traits in natural populations of a tropical drosophilid: *Zaprionus indianus*. *Genetica* **108**, 91–100.

Misra R. K. and Reeve E. C. R. 1964 Clines in body dimensions in populations of *Drosophila subobscura. Genet. Res*. **5**, 240–256.

Moreteau B., Gibert P., Moreteau J. C., Huey R. B. and David J. R. 2003 Morphometrical evolution in a *Drosophila* clade: the *Drosophila obscura* group. *J. Zool. Syst. Evol. Res.* **41**, 64–71.

Morin J. P., Moreteau B., Pétavy G. and David J. R. 1999 Divergence of reaction norms of size characters between tropical and temperate populations of *Drosophila melanogaster* and *D. simulans. J. Evol. Biol*. **12**, 329–339.

Noach E. J. K., de Jong G. and Scharloo W. 1996 Phenotypic plasticity in morphological traits in two populations of *Drosophila melanogaster*. *J. Evol. Biol*. **9**, 831–844.

Partridge L. and Coyne J. A. 1997 Bergmann's rule in ectotherms: is it adaptative? *Evolution* **51**, 632–635.

Prevosti A. 1955 Geographical variability in quantitative traits in populations of *Drosophila subobscura. Cold Spring Harbor Symp. Quant. Biol*. **20**, 294–299.

Pétavy G., Morin J. P., Moreteau B. and David J. R. 1997 Growth temperature and phenotypic plasticity in two *Drosophila* sibling species: probable adaptive changes in flight capacities. *J. Evol. Biol.* **10**, 875–887.

Pétavy G., David J. R., Gibert P. and Moreteau B. 2001 Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. *J. Therm. Biol.* **26**, 29–39.

Pétavy G., David J. R., Debat V., Gibert P. and Moreteau B. 2004 Specific effects of cycling stressful temperatures upon phenotypic and genetic variability of size traits in *D. melanogaster. Evol. Ecol. Res.* **6**, 873–890.

Peters R. H. 1983 *The ecological implications of body size*. Cambridge University Press, Cambridge.

Polak M. 2003 *Developmental instability: causes and consequences*. Oxford University Press, Oxford.

Reiss M. J. 1989 *The allometry of growth and reproduction*. Cambridge University Press, Cambridge.

Rutherford S. L. and Lindquist S. 1998 Hsp90 as a capacitor for morphological evolution. *Nature* **396**, 336–342.

Sokal R. R. and Rohlf F. J. 1995 *Biometry: the principles and practice of statistics in biological research*, 3rd edition. Freeman, New York.

Stalker H. D. 1980 Chromosome studies in wild populations of *Drosophila melanogaster*. II. Relationship of inversion frequencies to latitude, season, wing-loading and flight activity. *Genetics* **95**, 211–223.

Stalker H. D. and Carson H. L. 1947 Morphological variation in natural populations of *Drosophila robusta* Sturtevant. *Evolution* **1**, 237–248.

StatSoft (1999) *Statistica* Version 5.5. StatSoft, Inc., Tulsa, USA.

van der Have T. M. and de Jong G. 1996 Adult size in ectotherms: temperature effects on growth and differentiation. *J. Therm. Biol*. **183**, 329–340.

Van Voorhies W. A. 1996 Bergmann size clines: a simple explanation for their occurrence in ectotherms. *Evolution* **50**, 1259–1264.

Zhang X. S. and Hill W. G. 2005 Evolution of the environment component of the phenotypic variance: stabilizing selection in changing environments and the cost of homogeneity. *Evolution* **59**, 1237–1244.

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