Original article

Reaction norms of size characters in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis

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Summary – Ten isofemale lines of *Drosophila melanogaster*, recently collected in a French vineyard, were submitted to 7 different developmental temperatures, from 12 to 31°C, encompassing the whole physiological range of the species. For each line and temperature, 10 flies of each sex were collected randomly and 2 size-related traits were measured: wing and thorax length. Both traits exhibited similar response curves: a maximum size at a low temperature and a decrease on both sides. ANOVA showed significant variations between lines and also significant line–temperature interactions, demonstrating different norms of reaction among the various lines. The shapes of the curves were further analysed by considering slope variations, *ie* by calculating empirical derivative curves. The most interesting observation is that the temperature of maximum size (TMS) is not the same for the wing (average 15.73 ± 0.29°C) and the thorax (average 19.57 ± 0.47°C). Genetic differences seem to exist between lines, and TMS for both traits are correlated. Sexual dimorphism was analysed by considering the female/male ratio for wing and thorax. Both traits provided the same information: sexual dimorphism increased, from 1.10 to 1.16, with increasing temperature, and significant differences were found between lines. Finally the wing/thorax ratio appeared as an original and most interesting trait. This ratio, which is less variable than wing or thorax, exhibited a monotonously decreasing sigmoid shape, from 2.80 to 2.40, with increasing temperature. It is suggested that this ratio, which may be related to flight capacity at various temperatures, could be the direct target of natural selection.

reaction norm / wing length / thorax length / developmental temperature / sex dimorphism / wing/thorax ratio / flight capacity

Résumé – Normes de réaction de caractères de taille chez *Drosophila melanogaster* en fonction de la température de développement : une analyse de lignées isofemelles. *Dix lignées isofemelles de Drosophila melanogaster, récemment récoltées dans un vignoble français du sud-ouest de la France, ont été soumises à 7 températures différentes (de 12 à 31°C) compatibles avec le développement de l'espèce. Pour chaque lignée et chaque température, 10 mouches de chaque sexe ont été choisies au hasard. Sur chaque individu, 2 caractères relatifs à la taille ont été mesurés : la longueur de l'aile et la longueur du thorax. Les courbes de réponse des 2 caractères ont la même forme et mettent en évidence une taille maximum en dessous de 20°C et une décroissance de part et d'autre de ce maximum. Des variations significatives entre les lignées de même que des interactions significatives lignée–température sont mises en évidence par ANOVA, ce qui montre que les normes de réaction des différentes lignées ont des formes différentes. L'analyse de la forme des courbes a été réalisée en considérant les variations des pentes pour chaque intervalle de température, c'est-à-dire en calculant empiriquement une dérivée. L'observation la plus remarquable concerne la température pour laquelle la taille est maximale:* $15,73 \pm 0,29°C$ *pour l'aile et* $19,57 \pm 0,47°C$ *pour le thorax. Des différences génétiques entre les lignées sont mises en évidence pour cette température de taille maximum, et les valeurs obtenues pour les 2 caractères sont corrélées. Le rapport femelle–mâle pour l'aile ou le thorax permet d'étudier le dimorphisme sexuel. Le rapport augmente de 1,10 à 1,16 quand la température passe de 12 à 31°C. Il existe aussi des différences significatives entre les lignées. Il est montré que le rapport aile-thorax est un critère original et d'un grand intérêt. Ce rapport est relativement moins variable que l'aile ou le thorax. Il décroît selon une sigmoïde à mesure que la température augmente et varie de 2,80 à 2,40. Vraisemblablement en relation avec la capacité de vol en fonction de la température, le rapport aile-thorax pourrait être la cible directe de la sélection naturelle.*

normes de réaction / longueur de l'aile et du thorax / température de développement / dimorphisme sexuel / rapport aile–thorax / capacité de vol

INTRODUCTION

For ectothermic organisms, like *Drosophila*, temperature is the most important abiotic factor for explaining the geographic distribution and abundance of species (David *et al*, 1983; Parsons, 1983; Hoffmann and Parsons, 1991). Among more than 20 species that now exhibit a cosmopolitan status, only 2 (*D melanogaster* and *D simulans*) were able to adapt to different climates and proliferate both in temperate and tropical regions (David and Tsacas, 1981). Various species, including *D subobscura, D robusta, D melanogaster* and *D simulans* (see David *et al*, 1983; Capy *et al*, 1993), exhibit genetic latitudinal clines for their size, and flies are larger at higher latitudes. Also laboratory experiments made on *D pseudoobscura* (Anderson, 1966), *D willistoni* (Powell, 1974) and more recently on *D melanogaster* (Cavicchi *et al*, 1985) have described a genetically determined increase in size by keeping populations at a low temperature for many generations, and an opposite effect with high temperatures. From these convergent observations, little doubt remains that a colder environment favors a larger size, and *vice versa*, although we do not have up to now a plausible interpretation for this interaction.

The problem becomes still more complicated if we consider that size also exhibits a broad phenotypic plasticity which, in natural populations, is expressed by a high value of the standard deviation or the coefficient of variation of size characters (Atkinson, 1979; David *et al*, 1980; Coyne and Beecham, 1987).

Two kinds of environmental factors control adult size during development: larval nutrition and temperature. Among individuals collected at the same time, size differences are mainly due to nutritional effects, although some temperature variations may also occur. Thermal effects, on the other hand, are more important when different seasons are compared (Atkinson, 1979).

Natural size variations may be heritable (Coyne and Beecham, 1987). On the other hand, a positive correlation seems to exist between size and fitness in wild living males (Partridge *et al*, 1987) or females (Boulétreau, 1978). How a natural population keeps a stable size presumably implies trade-offs between fitness traits, but the precise mechanisms remain unknown.

From an ecophysiological point of view, the response curves of size characters (weight, lengths of various body parts) are broadly known (see David *et al*, 1983) and, when plotted against temperature on the X axis, exhibit the shape of an inverted U. Many points however remain insufficiently analysed and deserve further study. First, is there a genetic variability not for size itself, but for the shape of the curve, *ie* for what is now called the norm of reaction? Second, are there different norms between various morphological traits which are all related to size? Third, how can we interpret the norms of reaction in an evolutionary perspective? More precisely, which traits are specifically related to natural selection and adaptation, and which can be considered as contingent, *ie* related to internal genetic constraints?

In the present paper, variations of 2 size characters (wing and thorax) have been considered in relation to growth temperature. Genetic variations of the norms of reaction were analysed by comparing 10 isofemale lines. The norms of reactions of wing and thorax, although similar, are not identical, and especially the temperatures of maximum size are different. Moreover, these parameters exhibit genetic variations which are correlated for wing and thorax. The adaptive significance of the shape of the response curves is not obvious, although the wing/thorax ratio could be more interesting in this respect. The norm of reaction of this trait is more simple since we found a regularly decreasing curve with increasing temperature. We suggest that this ratio, or some other related parameter, could be the immediate target of natural selection, in relation to the flight capacity at different temperatures.

MATERIALS AND METHODS

Flies from a wild living vineyard population were collected with banana traps in the Grande Ferrade estate, in Pont-de-la-Maye, near Bordeaux. About 20 females were isolated in culture vials (cornmeal medium with live yeast) and produced a first laboratory generation, G1, grown at 25°C. Ten lines were then randomly chosen to produce the experimental flies. For this, 10 females and 10 males from each G1 line were used as parents. They oviposited at 20°C on a killed yeast, high nutrient medium (David and Clavel, 1965) for about half a day. Vials with eggs were then

transferred at 1 of the 7 experimental constant temperatures, *ie* 12, 14, 17, 21, 25, 28 and 31°C. With this procedure larval density was not strictly controlled, and the number of adults emerging from a vial generally ranged between 100 and 200. This is a fairly high density. On the other hand, the use of a very rich medium for the development prevented significant crowding effects which often result in a decrease in fly size.

For each temperature and line, we used only a single culture vial. A long experience with the technique has shown that variations due to vial differences (*ie* common environment effects) are negligible. On the other hand, the occurrence of such effects would increase the error variation and make genetic differences (*eg*, between lines) more difficult to demonstrate.

From each line at each temperature, 10 females and 10 males were randomly chosen and studied. On each fly 2 traits were measured with an ocular micrometer in a binocular microscope: wing length with a 25 × magnification and thorax length with a 50 × magnification. In the *Results* section lengths are expressed in hundreths of mm, *ie* micrometer units were multiplied by 2 for the thorax and by 4 for the wing.

Thorax length was measured on a left side view, from the anterior margin at the neck level to the tip of the scutellum. For wing length a difficulty exists in defining the anterior basis of the wing. We used the middle part of the thoracic coast, in front of the tegula, since we found it easier to identify this point with accuracy on a lateral view. For the posterior part we used the tip of the wing at the end of the third longitudinal vein.

Statistical analyses, and especially analysis of variance (ANOVA), were done with SAS (SAS Institute Inc, 1985). Temperature, lines and sex were considered as fixed effects.

RESULTS

We will first consider wing and thorax length, and in a second section, the wing/thorax ratio, which appeared to be an original and interesting trait. The illustrations deal either with lengths or with the ratio. In the tables, however, we often include simultaneous analyses concerning wing, thorax and ratio, in order to save space. Data included in the tables but concerning the ratio is discussed in the second section.

Wing and thorax length

Average response curves

The average response curves are shown in figure 1. Female and male curves are separated, showing the well-known fact that males are smaller than females. The norms of reaction of the 2 traits have quite similar shapes, confirming previous results (David *et al*, 1983). A maximum size is observed at a fairly low temperature, around 15°C for the wing and 19°C for the thorax. A significant decrease is observed on both sides of this maximum, *ie* higher or lower temperatures.
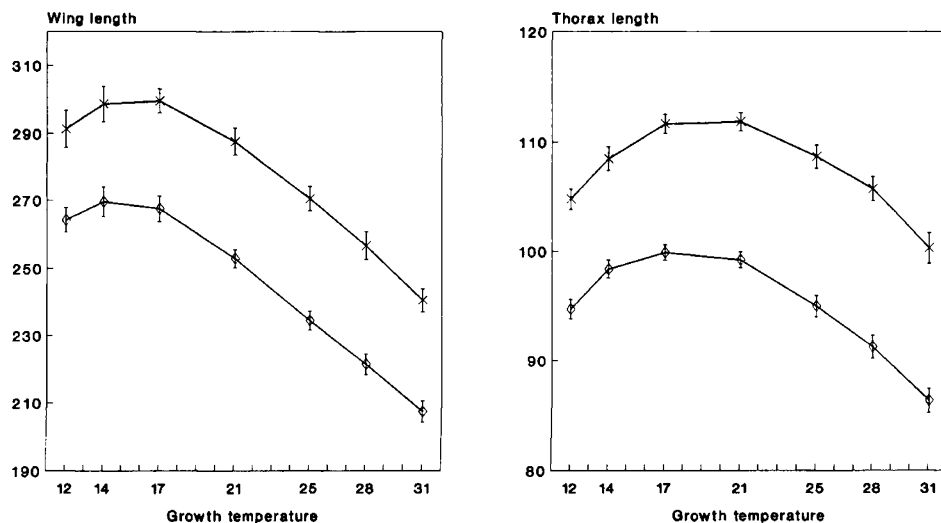
Fig 1. Variation of wing or thorax length according to growth temperature. Lengths are expressed in mm × 100. Vertical bars indicate the confidence interval of the mean (2 se). Female: × ; male: ◊.

Sources of variation

The data shown in figure 1 were submitted to ANOVA, in order to identify the significant sources of variation, and the results are given in table I. The main variations are due to sex and temperature. A highly significant line effect due to genetic differences is also observed. All the double interactions are highly significant, while the triple interaction is not. The line × temperature interaction means that the norms of reaction of the various lines are not parallel and exhibit different shapes. The sex × line interaction means that there is some sexual dimorphism in the norms of reaction.

Within-line variability

This variability deserves further attention. We may ask 2 related questions: does variability change with temperature, and are some lines more variable than others? In this analysis, we have considered 2 parameters, the standard deviation and the CV (coefficient of variation), and the results are shown in figure 2.

   Standard deviations are much higher for the wing than for the thorax. For the wing, a decrease in the standard deviation is observed with increasing temperature, as well as a lower value in males. Some of these differences may be due to the fact that the wing is about 2.5 times longer than the thorax, and that males are smaller than females. To avoid this scaling effect, we used a relative measurement, the CV. Of course, each CV was calculated on a group of 10 flies (same line and temperature) so that the total number of observations is 140 for 1 400 individuals. As seen in figure 2, the relative variability is about the same for males and females,

Table I. Results of ANOVA on wing and thorax length and wing/thorax ratio.

Source of variation	Degree of freedom	Wing		Thorax		Ratio	
		MS	F	MS	F	MS ($\times 10^3$)	F
Sex	1	368 972.80	12 785.63***	53 395.23	12 971.00***	19.81	13.91***
Line	9	4 310.60	149.37***	194.46	47.24***	173.33	121.71***
Temperature	6	111 243.84	3 854.82***	3 879.38	942.40***	4 884.00	3 429.41***
Sex × line	9	159.36	5.52***	13.74	3.34***	3.16	2.22*
Sex × temperature	6	557.92	19.33***	163.01	39.60***	7.35	5.16***
Line × temperature	54	127.68	4.43***	19.51	4.74***	7.06	4.96***
Sex × line × temperature	54	29.28	1.01ns	4.51	1.09ns	2.27	1.59**
Residual	1 260	28.86		4.12		1.42	

MS: mean square; F: variance ratio; significance: ns non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
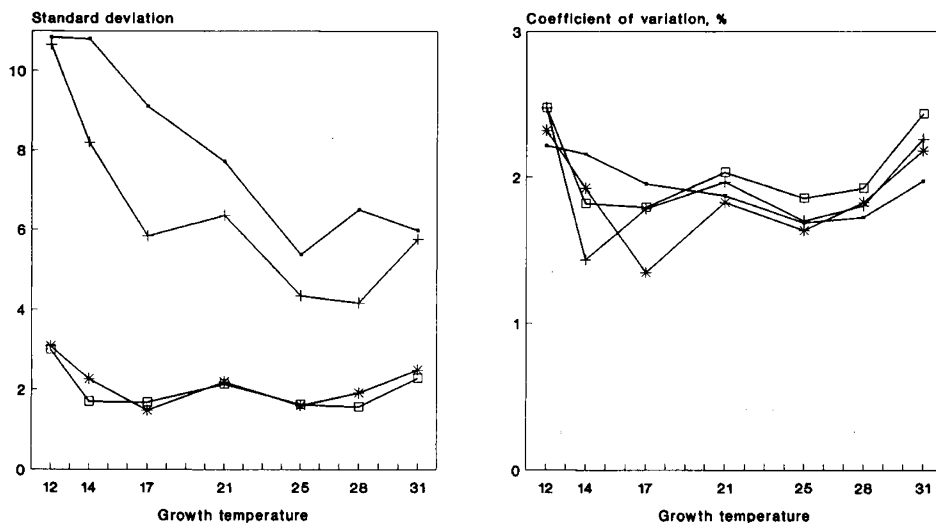
Fig 2. Change in individual variability of wing or thorax length according to growth temperature. Left: variation of standard deviations; right: variation of coefficients of variation. ● Wing female; ∗ thorax female; + wing male; □ thorax male.

and is also similar for both traits. These data were submited to ANOVA (table II) and the conclusion was significant effects for temperature in both traits, while line differences ($p = 0.011$) and sex ($p = 0.016$) were significant only for the thorax. None of the interactions were significant. Concerning the temperature effect (see figure 11 below) we note a relative stability of the CV at intermediate temperatures and an increase at extreme temperatures, especially at 12 and 31°C.

Between-line variability and intraclass correlations

Variation between lines is illustrated in figure 3. The significant line × temperature interaction is visualized on the graph by the intercrosses of the lines.

For each temperature, the between-line variance was calculated, and also the coefficient of intraclass correlation which estimates an 'isofemale line heritability' (Hoffmann and Parsons, 1988). Results are shown graphically in figure 4 and analysed with ANOVA in table III.

For wing length, no effects are significant, and the mean values are 0.58 ± 0.03 and 0.51 ± 0.03 for females and males, respectively. The picture is different for the thorax: males have significantly lower values than females (0.30 ± 0.04 against 0.37 ± 0.04) and variations occur according to temperature (see figure 4). More precisely, intraclass correlation is higher at high temperatures (25–31°C) than at low temperatures (12–21°C). A last conclusion is that the overall genetic variability is much less for the thorax than for the wing.

Table II. Results of ANOVA on the coefficients of variation of wing and thorax length and wing/thorax ratio.

Source of variation	Degree of freedom	Wing		Thorax		Ratio	
		MS	F	MS	F	MS ($\times 10^3$)	F
Sex	1	2.65	0.72^{ns}	3.43	6.15^*	0.05	1.06^{ns}
Temperature	6	16.88	4.56^{***}	6.31	11.33^{***}	0.9	17.50^{***}
Line	9	3.38	0.91^{ns}	1.51	2.71^*	0.3	5.33^{***}
Sex × temperature	6	2.23	0.60^{ns}	0.35	0.63^{ns}	0.06	1.14^{ns}
Sex × line	9	2.56	0.69^{ns}	0.54	0.97^{ns}	0.05	1.06^{ns}
Temperature × line	54	3.76	1.02^{ns}	0.85	1.54^{ns}	0.08	1.69^*
Residual	54	3.70		0.56		0.05	

Each basic observation is calculated on 10 flies; MS: mean square; F: variance ratio; significance: ns non-significant; * $p < 0.05$; *** $p < 0.001$.
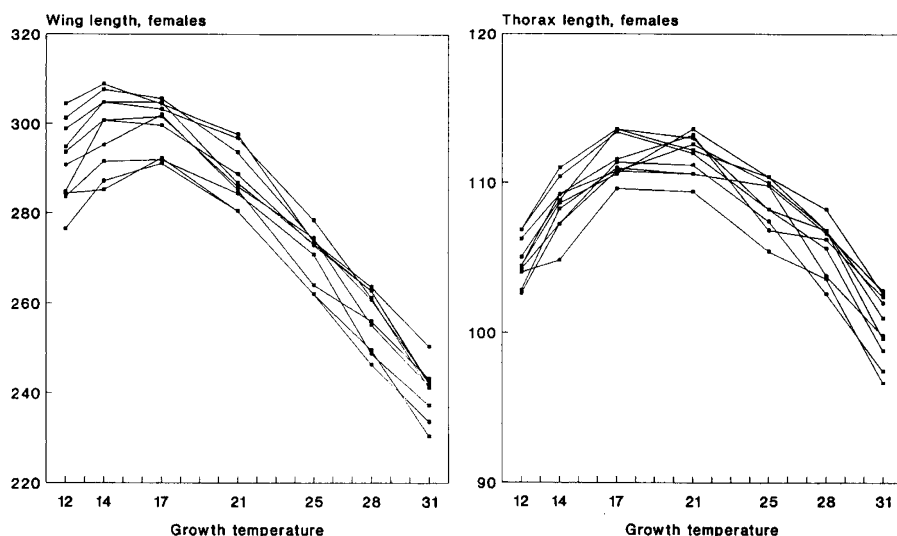
Fig 3. Presentation of wing and thorax length variations of females in the 10 isofemale lines. For clarity, the standard errors of each point are not shown.

Between-sex correlation and sex dimorphism

Previous analyses have already evidenced numerous sex differences and sex interactions with other factors. In this section we consider correlations between sexes at the same temperature, and also the female/male ratio.

Male–female correlations can only be analysed by considering the mean values of each line. The results are shown in table IV. The average correlation is higher for the wing (0.91) than for the thorax (0.76). This is significant if we consider the average difference over temperatures ($d = 0.144 \pm 0.054$, $t = 2.66$, $n = 7$).

Sexual differences, for each line and each temperature, were examined by calculating the female/male ratio. Results of ANOVA are given in table V. For both traits, temperature and line effects are significant. Heritable variations occurred between lines. The temperature effects are shown in figure 5. Both traits show the same pattern: the female/male ratio decreases regularly from high temperatures (1.16) to low temperatures (1.10). The 2 sexes are more similar when grown at low temperature.

Finally, the relationship between the sexual dimorphism of wing and thorax was investigated by calculating the correlation at each temperature. The mean value for the 7 temperatures ($r = 0.67 \pm 0.07$) is clearly positive and significant: sexual dimorphism is higher in some lines than in others.

Intraclass correlation, males
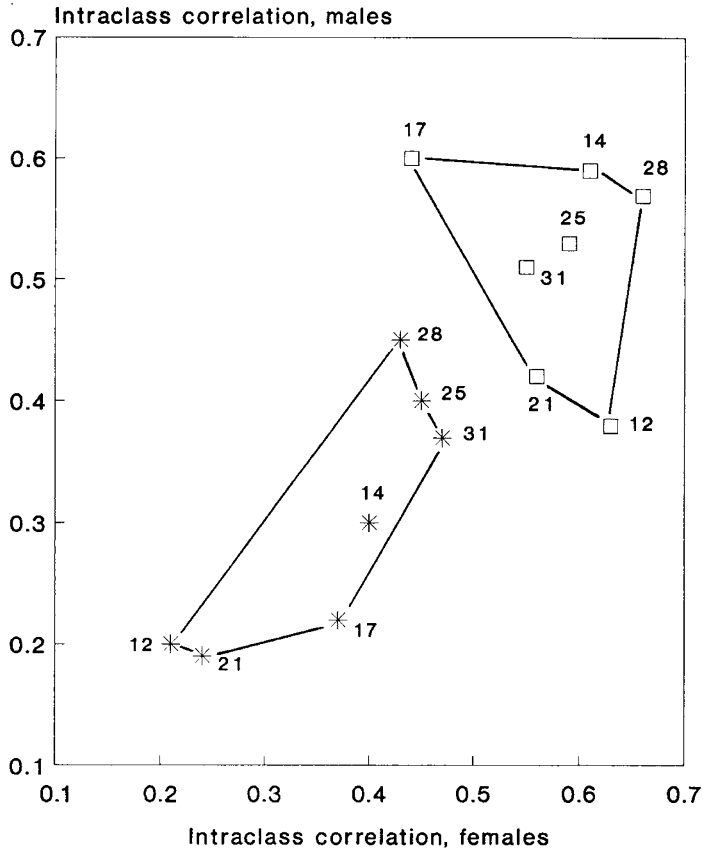
Fig 4. Intraclass correlations (isofemale heritability) for wing or thorax length at various temperatures. The temperature of development is indicated for each point. □ Wing; * thorax.

Shape of the norms of reaction: variation of the slope and derivative curves

For each isofemale line, the size variation for a given temperature interval allows the calculation of a slope (*ie* size variation for one degree change) if we accept a linear intrapolation. When this operation is repeated over successive temperature intervals, we get an empirical derivative of the norm of reaction. Examples of such curves are given, for females only, in figure 6. For both traits, the slope is monotonously decreasing from positive to negative values. The point where the curve crosses the zero line indicates the temperature of maximum size (TMS). As seen in figure 6, some variations exist for the same trait between lines, but there is no overlap for wing and thorax, as the ranges are 14.5–17°C and 18–21°C, respectively.

Table III. Results of ANOVA on the coefficients of intraclass correlation (isofemale lines heritability) for wing and thorax length and wing/thorax ratio.

Source of variation	Degree of freedom	Wing		Thorax		Ratio	
		MS	F	MS	F	MS	F
Sex	1	0.0138	1.77^{ns}	0.0138	8.16^{*}	0.000007	0.001^{ns}
Temperature	6	0.0045	0.58^{ns}	0.0198	11.69^{**}	0.00413	0.75^{ns}
Residual	6	0.0078		0.002		0.005	

MS: mean square; F: variance ratio; significance: ns non-significant; * $p < 0.05$; ** $p < 0.01$.

Table IV. Pearson's coefficients of correlation between male and female values of the 10 isofemale lines at each temperature, for wing and thorax length and wing/thorax ratio.

|	$12°C$	$14°C$	$17°C$	$21°C$	$25°C$	$28°C$	$31°C$	m
Wing	0.92	0.92	0.96	0.87	0.92	0.82	0.94	0.91 ± 0.05
Thorax	0.50	0.88	0.78	0.70	0.89	0.65	0.94	0.76 ± 0.16
Ratio	0.76	0.88	0.87	0.91	0.93	0.88	0.87	0.87 ± 0.05

m: mean value

Table V. Results of ANOVA on sexual dimorphism (female/male ratio for each line) of wing and thorax length and wing/thorax ratio.

Source of variation	Degree of freedom	Wing		Thorax		Ratio	
		MS	F	MS	F	MS	F
Temperature	6	0.095	54.1^{***}	0.023	51.6^{***}	0.0002	2.9^{**}
Line	9	0.006	3.7^{***}	0.001	2.8^{***}	0.0001	1.2^{ns}
Residual	54	0.002		0.0004		0.0001	

MS: mean square; F: variance ratio; significance ns non-significant; ** $p < 0.01$; *** $p < 0.001$.

Statistical analyses are presented in table VI. Significant effects are due to temperature and sex, but not to lines. On the other hand, a significant line $\times$ temperature interaction is observed, which means that the derivative curves of the various lines have different shapes.

Figure 6 shows that variation in slope is much greater for wing than thorax (notice that the ordinate scales are not the same on the 2 graphs). However, as with the standard deviation, this may be due to a scaling effect related to the greater length of the wing. For a better comparison of the 2 traits, the standardized derivatives (slope-to-mean ratio) were calculated and the average curves are shown in figure 7. With this transformation the relative variabilities of the 2 traits are

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Female/male ratio

Growth temperature
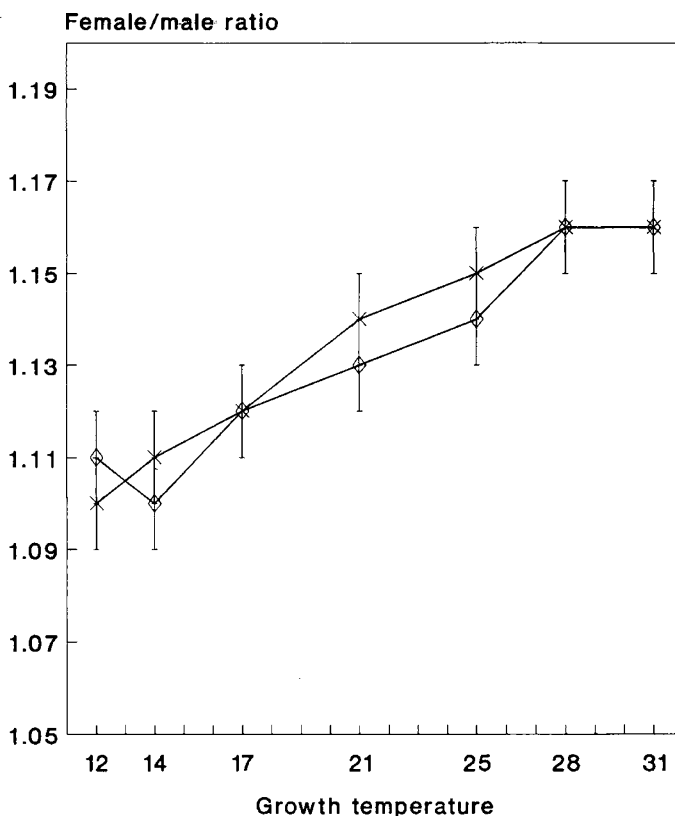
Fig 5. Variation of sexual dimorphism (female-to-male ratio) according to growth temperature. Data for wing (×) or thorax (◊) length.

comparable, and the main difference seems to be a translation of the thorax curves to the right, *ie* toward higher temperatures. A difference also exists between sexes, the female curves are also to the right of the male curves.

Temperature of maximum size (TMS)

As indicated in figure 6, the temperature at which the derivative is zero corresponds to the maximum size of the trait. For each line, the TMS was calculated by assuming a linear variation of the derivative between the lowest temperature and the 17–21°C interval for the wing, the 21–25°C interval for the thorax. More precisely, if we consider the average curves of figure 7, 3 points were used to calculate the TMS of the wing and 4 points for the thorax.
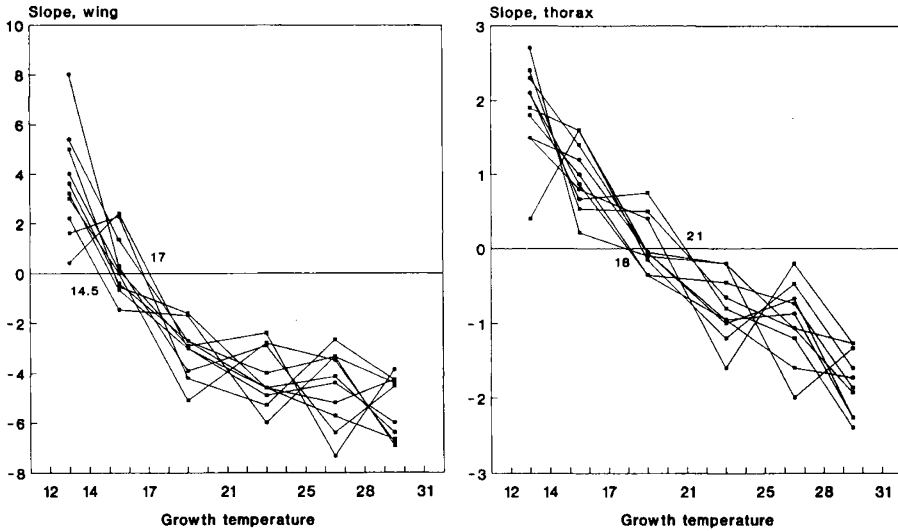
Fig 6. Variation of the slopes of reaction norms among females of 10 isofemale lines. These curves are the empirical derivatives of those shown in figure 3. Zero-value slopes indicate the TMS of each line. There is no overlap for TMS of wing and thorax.

Table VI. Results of ANOVA on the slopes of reaction norms for wing and thorax length and wing/thorax ratio.

Source of variation	Degree of freedom	Wing		Thorax		Ratio	
		MS	F	MS	F	MS	F
Sex	1	3.56	4.1*	4.52	11.6***	0.0002	0.31^{ns}
Temperature	5	209.6	240.9***	35.45	363.5***	0.0009	17.79***
Line	6	0.77	0.9^{ns}	0.13	1.32^{ns}	0.00004	0.7^{ns}
Sex × temperature	5	2.5	2.8*	0.29	3.02**	0.0002	3.6***
Sex × line	9	0.24	0.3^{ns}	0.04	0.44^{ns}	0.00003	0.55^{ns}
Line × temperature	45	2.9	3.3***	0.34	3.5***	0.0001	1.83**
Residual	45	0.87		0.098		0.0001	

MS: mean square; F: variance ratio; significance: ns non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Variations of the TMS are shown in figure 8, and mean values are given in table VII. ANOVA, applied to these data, demonstrated significant effects of traits, sex and lines.

The left-hand graph of figure 8 illustrates the large difference between the 2 traits, with no overlap between the distributions. Moreover, the positive correlation between sexes of the same line suggests a genetic basis. A better characterization of each line is obtained by averaging the TMS of both sexes, as done in figure 8 (right-hand graph). A clear correlation is observed between thorax and wing TMS:
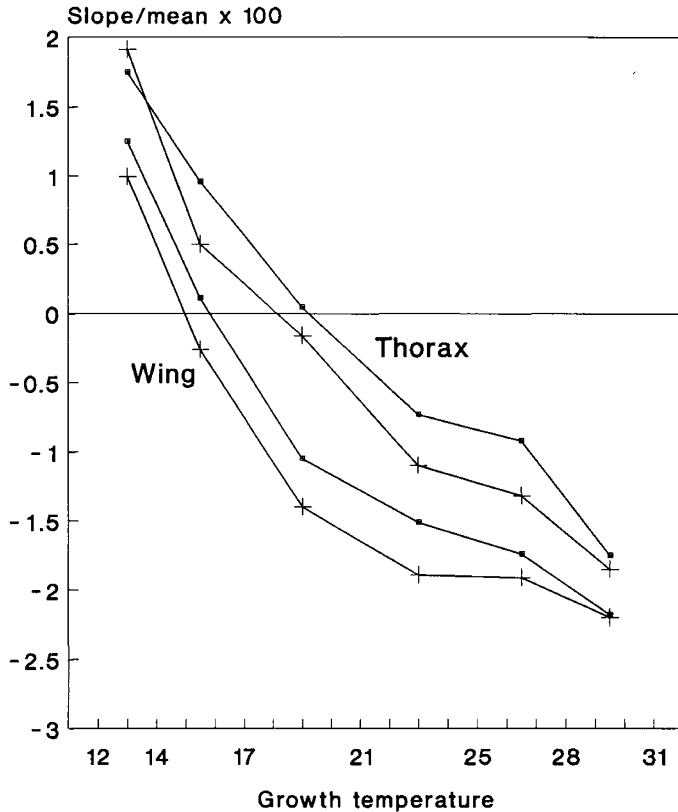
Fig 7. Average standardized derivative curves for wing and thorax lengths of both sexes. • Female; + male.

some lines exhibit a maximum size at a low temperature (around 14°C for the wing and 17°C for thorax); others a maximum size at higher temperatures (17°C for the wing and 21°C for the thorax).

Covariation of wing and thorax; the wing/thorax ratio

In the previous section, the relationship between wing and thorax variability was already considered in some cases, for example, for sexual dimorphism and TMS. In this section, we extend this investigation by considering the wing/thorax correlation and the wing/thorax ratio.

The wing/thorax correlation

The wing/thorax correlation may be investigated at the individual level (the 10 flies measured in each line and each temperature) or the line level (the 10 lines at each temperature). The coefficients of correlation are given in table VIII and their values

are quite stable over temperature. At the individual, within-line level, the values do not vary significantly according to temperature: the average phenotypic correlations are 0.71 for the females and 0.76 for males. In an extensive study, Scheiner *et al* (1991) found values at 19 and 25°C somehow higher (average 0.82). Genetic correlations found by Scheiner *et al* (1991) were a little lower (0.73 for females) in close agreement with the between-line correlations we found in the present study (table VIII).
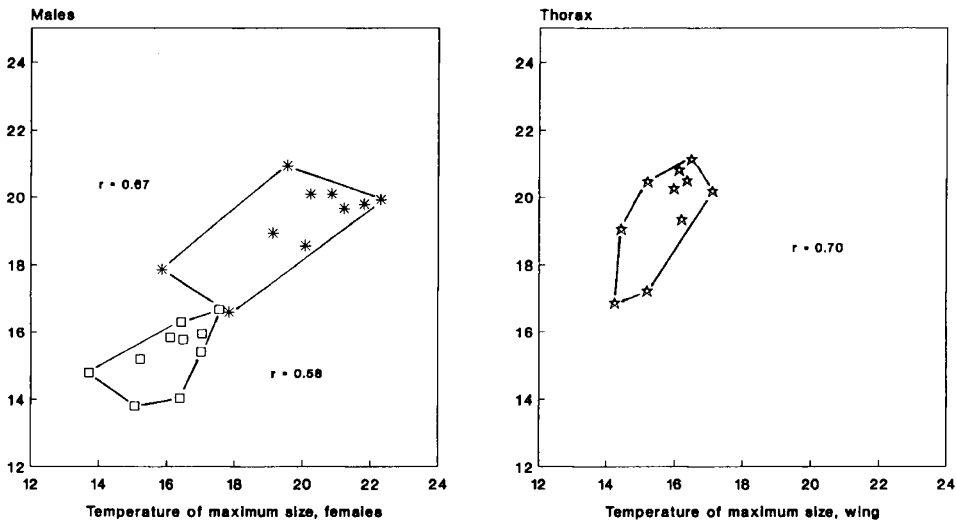
Fig 8. Analysis of the variability of TMS among isofemale lines. Left: relationship between TMS of males and females for wing (□) or thorax (*); right: relationship between TMS of wing and thorax (for each line, both sexes are averaged).

Table VII. Temperature of maximum size (mean ± se) for wing and thorax length.

|	*Females*	*Males*	*Both sexes*
Wing	16.10 ± 0.36	15.37 ± 0.29	15.73 ± 0.29
Thorax	19.89 ± 0.61	19.24 ± 0.40	19.57 ± 0.47

The wing/thorax ratio: mean values

During the development of our investigations, it turned out that the covariation of the wing and thorax could be investigated in an interesting way: by calculating the wing/thorax ratio. This trait, as well as the length, varies according to sex, temperature and lines (see table I) and moreover all interactions are significant.

Table VIII. Coefficients of correlation between wing and thorax lengths at various temperatures.

Temperature °C	Within lines		Between lines	
	Female	Male	Female	Male
12	0.66 ± 0.21	0.73 ± 0.17	0.89	0.70
14	0.67 ± 0.22	0.74 ± 0.17	0.85	0.80
17	0.69 ± 0.24	0.77 ± 0.11	0.70	0.74
21	0.76 ± 0.13	0.79 ± 0.10	0.77	0.65
25	0.75 ± 0.13	0.75 ± 0.12	0.68	0.77
28	0.70 ± 0.15	0.70 ± 0.11	0.86	0.77
31	0.77 ± 0.10	0.83 ± 0.06	0.80	0.69
m	0.71 ± 0.02	0.76 ± 0.02	0.78 ± 0.04	0.73 ± 0.02

Within-line correlations were calculated at the individual level and averaged over lines (mean ± se). Between-line correlations are calculated between the means of 10 lines; *m*: average over temperatures.
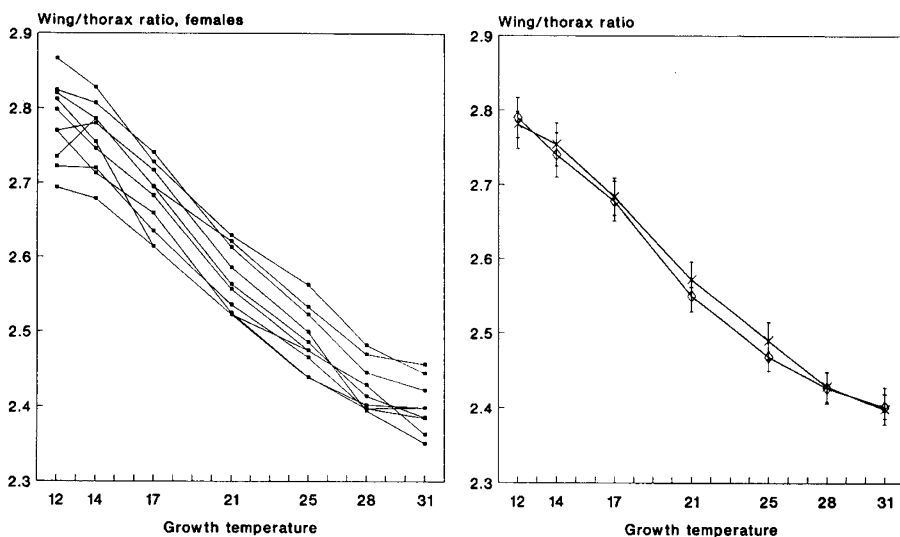
Fig 9. Variation of wing/thorax ratio according to growth temperature. Left: variability among 10 isofemales lines (female data); right: variation of mean values for males (◊) and females (×) (vertical bars indicate the confidence interval).

Variations are shown graphically either at the line level, or by considering the average values of each sex (fig 9).

The ratio exhibits a monotonic decrease from low temperatures (about 2.8) to high temperatures (about 2.4). Interestingly, male and female values are very close, although statistically different.

The wing/thorax ratio: slopes and derivative curves

For each line and temperature interval the slope was calculated. As an example, the female values are shown for the 10 lines in figure 10. Large variations exist between lines. With one exception, all values are negative, indicating that the ratio decreases with increasing temperature. In spite of the broad dispersal it is possible to conclude that the slope varies according to temperature (ANOVA, table VI). This is also illustrated by considering the confidence intervals of the mean values in figure 10. For example, the average slope between 17 and 21°C (-0.03) is much less than between 28 and 31°C (-0.01). Such variations of the derivatives demonstrate that the decrease of the wing/thorax ratio, illustrated in figure 9, is not a linear function of temperature, but a decreasing sigmoid.
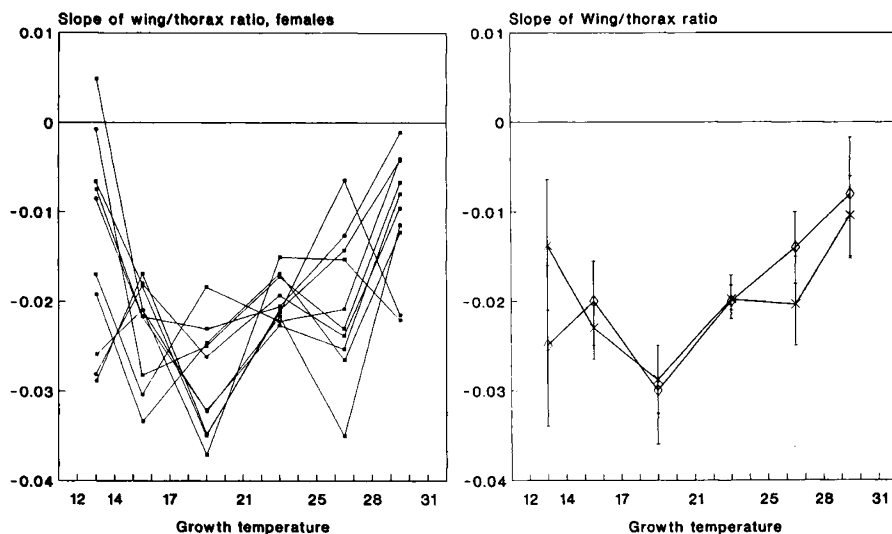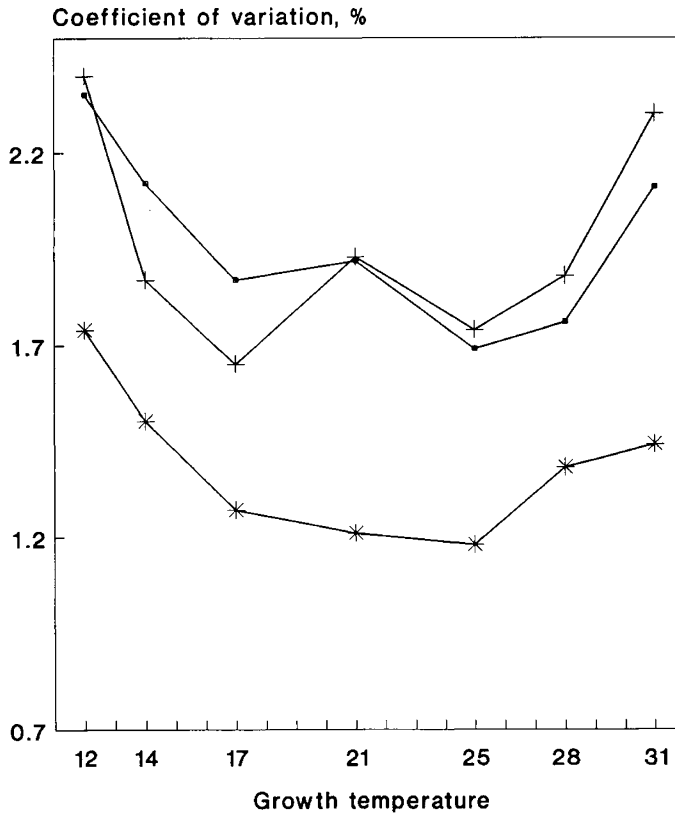

Fig 10. Variation of the slope of the wing/thorax ratio according to growth temperature. Left: variability among 10 isofemale lines (female data); right: average curves for males ($\diamond$) and females ($\times$) (vertical bars indicate the confidence interval).

Within-line variability of the wing/thorax ratio

As for the lengths, the variability of the ratio was investigated by considering a relative measurement, the coefficient of variation. Results of ANOVA (table II) demonstrated very significant effects of temperature and lines but no difference between sexes. Interestingly, genetic differences between lines are much more pronounced for the ratio than for the traits themselves. The temperature effect is illustrated in figure 11, and compared to the CV of wing and thorax. The overall shapes are similar and correspond to U-shaped curves. Variability is minimum at intermediate temperatures and increases toward extreme, cold or hot, temperatures. Moreover the ratio is much less variable than the length. Between 17 and 25°C it

averages about 1.2%, while it is around 1.8% for the wing or thorax length. Such a reduction of the relative variability of the ratio is a consequence of the positive correlation existing between wing and thorax (table VII).

Fig 11. Variation of the within-line variability (average coefficient of variation) according to growth temperature; both sexes averaged. Notice the increased variability at extreme temperature, and the lesser variability of the wing/thorax ratio. • Wing; + thorax; * ratio.

Between-line variability: intraclass correlation

The values of the coefficients of intraclass correlation for the wing/thorax ratio are given in table IX. Thera are no significant variations between sexes or temperatures. The overall mean, calculated on 14 observations, is 0.52 ± 0.02. The isofemale line heritability of the ratio is high, comparable to that of the wing and superior to that of the thorax.

Table IX. Coefficients of intraclass correlation for the wing/thorax ratio at various temperatures.

	12° C	*14° C*	*17° C*	*21° C*	*25° C*	*28° C*	*31° C*	m
Female	0.49	0.48	0.53	0.58	0.63	0.48	0.46	0.52 ± 0.02
Male	0.42	0.58	0.63	0.50	0.49	0.46	0.58	0.52 ± 0.03

m: mean value ± se

DISCUSSION

As pointed out in the *Introduction*, numerous observations and arguments suggest that size variations are strongly related to fitness and that size is a regular target of natural selection. On the other hand, the overall size is a difficult entity to define since measurements deal only with size-related traits. Wing and thorax length and adult weight are the most generally used traits (Capy *et al*, 1993) but other dimensions have also been considered in the literature, such as head width or the lengths of various parts of the legs. As far as we know (David *et al*, 1983), all these traits exhibit similar convex response curves to growth temperature, with a maximum below 20°C and a decline on both sides. A maximum size is often referred to as an 'optimum' (Gabriel and Lynch, 1992) but this must be considered cautiously (see David *et al*, 1983). In the present work, we demonstrated that temperatures of maximum length for wing and thorax are clearly separated, thus making the argument of an optimum size still more difficult.

In the *Drosophila* literature, many papers have dealt with the genetic architecture and heritability of wing or thorax length (reviewed in Roff and Mousseau, 1987). The isofemale line technique also provides an opportunity to estimate the intrapopulation variability with the coefficient of intraclass correlation (Hoffmann and Parsons, 1988). We have found that this 'isofemale line heritability' is higher for wing than thorax, thus confirming extensive data on numerous geographic populations (Capy *et al*, 1994). More interesting is the fact that, for the thorax only, this heritability seems to vary according to the environment, being higher at high temperatures.

As pointed out several times (De Jong, 1990; Falconer, 1990; Scheiner and Lyman, 1989, 1991; Scheiner, 1993), phenotypic plasticity may be considered as a specific trait, independent of the mean. Most empirical and theoretical analyses have considered only linear variations evaluated by considering 2 environments. For example, Scheiner and Lyman (1989, 1991) studied thorax length at 19 and 25°C and found that the heritability of plasticity was much less than that of thorax length. In a recent paper, Gavrilets and Scheiner (1993) have suggested a model for investigating nonlinear norms, and indicated the need for empirical, extensive data.

Wing and thorax changes are obviously nonlinear when studied over a broad range of temperatures. Such was also the case for pigmentation (David *et al*, 1989). Presumably, most morphometrical traits exhibit nonlinear norms, thus complicating mathematical analyses and theoretical interpretations. In the present work, we analysed the shapes of the response curves by calculating empirical derivatives,

as has been done previously for pigmentation (David *et al*, 1989). This technique is most convenient for comparing different traits. In this study, it enabled a precise calculation, for each trait, of the TMS. The facts that for each trait, the male and female TMS are correlated and also that, among lines, the thorax and wing TMS are also correlated, are strong arguments for assuming a genetic basis to these variations. Some lines have a maximum size at lower temperature, others at higher temperature: TMS itself is a trait which could be selected with the isofemale line technique as already used by Scheiner and Lyman (1991).

In *Drosophila*, as in most insect species, females are known to be bigger than males, although their development is faster (David *et al*, 1983). We estimated the sexual dimorphism by calculating, for each trait, the female/male ratio. Our data, based on the mean values of isofemale lines, led to several interesting conclusions. First, wing and thorax lengths provide about the same information on dimorphism. Second, sexual dimorphism increases with growth temperature, from a low 1.10 at 12°C to a high 1.16 at 28–31°C. Third, genetic variations exist between lines so that sexual dimorphism could also be selected.

Another ratio, which turned out to be very interesting, is the wing/thorax ratio, calculated either at the individual or line levels. The significance of this ratio for biometrical studies was already pointed out in recent papers on *D buzzatii* (Robertson, 1987; Thomas, 1993). Since, at the individual level, wing and thorax are correlated (r is about 0.70), the ratio is less variable than the traits themselves.

Comparing the variability of various traits having different means needs a relative measurement, *ie* the coefficient of variation (CV). Relative variabilities of wing and thorax are low and similar with an average value of 1.8% at medium temperatures. An increase which is found at extreme (low or high) temperatures may be considered as an increase of the developmental noise under stressful conditions. Indeed, a low relative variability may be considered as an indication of a physiological optimum (see David *et al*, 1983, for discussion). A long-standing argument is that fitness-related traits should exhibit a low variability due to genetic homeostasis (Lerner, 1954) and developmental canalization (Waddington, 1957). The fact that the ratio is still less variable (1.2%) than the traits themselves is mathematically due to the significant correlation existing between the 2 traits. On the other hand, the occurrence and persistence of this correlation in laboratory grown flies may be interpreted as a consequence of an internal constraint. This constraint could itself be an indication of a relationship between fitness and the wing/thorax ratio. Another argument is the overall shape of the reaction norm, *ie* a decreasing sigmoid curve. In this respect, there is a clear analogy with the abdominal pigmentation of the last 3 segments (David *et al*, 1989) for which an adaptive significance, related to the thermal budget, is likely. For the wing/thorax ratio, a possible direct adaptive significance, related to the flight capacity, may be proposed (Pétavy *et al*, 1992). For a given fly, the wing beat frequency increases with increasing ambient temperature (Reed *et al*, 1942) presumably due to a better muscle efficiency. On the other hand, a higher beating frequency should allow an increase of the wing loading, *ie* the weight per surface unit of the wing. In preliminary experiments, we measured the wing loading of males grown at 3 temperatures, *ie* 12, 21 and 30°C: average wing loadings were 0.24, 0.29 and 0.32 mg/mm^2, respectively. Wing beat frequencies were also measured, for these 3 categories of males, at 21°C, and a significant

increase from 195 to 247 Hz was observed with growth temperature, parallel to the morphological increase of the wing loading. Further more extensive studies are on the way. These preliminary data suggest that the morphological variations related to growth temperature are an adaptation to flying in a cold environment, with a lesser muscular efficiency and thus a decreased wing loading. We also found that the wing/thorax ratio is strongly correlated to wing loading. Interestingly, a similar decrease of the ratio with growth temperature has also been described in *D buzzatii* (Thomas, 1993). This could be a general feature in *Drosophila*.

A last problem, which has been discussed several times, is the relationship between plasticity and natural selection (David *et al*, 1983; Schlichting, 1986; Sultan, 1987; De Jong, 1989; Gabriel and Lynch, 1992; Gavrilets and Scheiner, 1993). Under the adaptive hypothesis, we should make 2 predictions. First, phenotypic plasticity itself could be selected for in populations and species living in a variable environment, for example, *Drosophila* populations in temperate countries which experience big and predictable seasonal thermal variations. On the other hand, for populations living in the thermal stability of tropical countries, plasticity should be a contingent property without a direct relationship to fitness. A second prediction is that, if the change in the mean phenotype is adaptive (*eg*, smaller flies in a warm environment) we should find a genetic modification when the environment is stable for many generations. We already know that prolonged culture at a higher temperature resulted in a decrease in size (Cavicchi *et al*, 1985) and also that tropical populations of *D melanogaster* have a smaller size (David and Capy, 1988). We now argue that the wing/thorax ratio (or some related measurement) could be the direct target of natural selection. If such is the case, a lower ratio should be found in tropical populations.

ACNOWLEDGMENTS

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