

Body size and developmental temperature in *Drosophila simulans*: comparison of reaction norms with sympatric *Drosophila melanogaster*

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(Received 10 November 1995; accepted 7 July 1996)

Summary – Reaction norms of two size-related traits (wing and thorax length) were analyzed in relation to growth temperature in a French natural population of *Drosophila simulans*, using the isofemale lines method. The wing/thorax ratio was also studied. Data were compared to those of the sibling species *Drosophila melanogaster* from the same locality. Flies were reared at seven constant temperatures, representing the whole thermal range of the two species. Phenotypic and genetic variabilities were analyzed. For investigating the shape of the response curves (ie, reaction norms) two methods were used: analysis of slope variations and polynomial adjustments. As expected from the relatedness of the two species, many similarities were observed. Notably, the reaction norms of wing and thorax lengths exhibited a maximum at low temperature, while the wing/thorax ratio was a regularly decreasing sigmoid curve. Numerous and sometimes great differences were also observed. At the phenotypic level, *D simulans* was generally more variable, while at the genetic level, it was less variable than *D melanogaster*. Isofemale line heritabilities varied according to growth temperature, but with different patterns in the two species. In both species, sexual dimorphism increased with temperature, but the average values and the response curves were different. The reaction norms of wing and thorax lengths were mainly characterized by different TMSs (temperatures of maximum size) with lower values in *D simulans*. This species was also characterized by a much lower wing/thorax ratio with a higher TIP (temperature of inflexion point). The possible adaptive significance of these variations remains unclear. Indeed, TMS variations suggest that *D simulans* could be more tolerant to cold than its sibling. On the other hand, the lower wing/thorax ratio of *D simulans* suggests a warm-adapted species.

phenotypic plasticity / isofemale line / wing length / thorax length / wing/thorax ratio

Résumé – Taille corporelle et température de développement chez *Drosophila simulans* : comparaison des normes de réaction avec l'espèce sympatrique *Drosophila melanogaster*. Les normes de réaction de la taille du corps (aile et thorax) et du rapport aile/thorax ont été analysées en fonction de la température de développement par la méthode des lignées isofemelles. Deux populations naturelles sympatriques françaises des espèces sœurs *Drosophila simulans* et *Drosophila melanogaster* ont été comparées. Les drosophiles ont été élevées à sept températures constantes comprises entre 12 et 31 °C, ce qui recouvre l'ensemble de la gamme des températures possibles pour ces deux espèces. La variabilité phénotypique entre les individus d'une même lignée a été analysée en utilisant les coefficients de variation, et la variabilité génétique en utilisant les coefficients de corrélation intraclasse. La forme des courbes de réponse (ie, normes de réaction) a été analysée par deux méthodes : la variation des pentes et les ajustements polynomiaux. En accord avec la parenté des deux espèces, de nombreuses similitudes ont été observées. En particulier les normes de réaction de l'aile et du thorax présentent un maximum à basse température, tandis que le rapport aile/thorax est une courbe sigmoïde décroissante. De nombreuses différences ont aussi été observées, parfois très importantes. Au niveau phénotypique, *D simulans* est généralement plus variable que *D melanogaster*, tandis qu'au niveau génétique elle s'est avérée en général moins variable. L'héritabilité varie avec la température, mais avec des modalités différentes dans chaque espèce. Dans les deux espèces, le dimorphisme sexuel (évalué par le rapport femelle/mâle) augmente avec la température, mais les valeurs et les courbes de réponse sont différentes. Les normes de réaction de l'aile et du thorax sont principalement différenciées par les TTMs (températures de taille maximale), avec des valeurs plus basses chez *D simulans*. Cette espèce est également caractérisée par un rapport aile/thorax inférieur avec une TPI (température de point d'inflexion) plus élevée. Ces différences sont difficiles à interpréter. En effet, les variations de TTMs suggèrent que *D simulans* pourrait être plus résistante au froid que *D melanogaster* ; en revanche le rapport aile/thorax plus faible de *D simulans* suggère une adaptation à la chaleur.

plasticité phénotypique / lignée isofemelle / taille de l'aile / taille du thorax / rapport aile/thorax

INTRODUCTION

Body size, which exhibits huge variations among living organisms, has long exerted a kind of fascination upon biologists. Size variations influence numerous biological traits, such as basal metabolism, duration of development or age at maturity (Reiss, 1989; Stearns, 1992; Charnov, 1993). Reciprocally, size is a target for natural selection and varies as a consequence of environmental pressures. For example, the old Bergman's rule describes, in numerous homeotherm species, an increase of size related to a colder environment. Finally size exhibits large variations between individuals of the same population, not only due to genetic differences but also due to phenotypic plasticity, related to different environmental conditions during development.

In *Drosophila*, allometric relationships are not well documented, although important size variations exist between species (Ashburner, 1989). Several species including *Drosophila melanogaster* and *Drosophila simulans* exhibit genetic latitudinal clines with a larger size under colder climate (David et al, 1983; Capy et al, 1993), these clines presumably being linked to temperature. Laboratory experiments keeping strains at different temperatures for many generations have demonstrated

genetic size variations over time, ie, smaller flies at high temperatures and bigger ones at low temperatures (Powell, 1974; Cavicchi et al, 1985; Partridge et al, 1994). These observations remind one of Bergman's rule, although *Drosophila* is an ectotherm so that we do not know why it should be better to be larger in a colder climate (David et al, 1994; Partridge et al, 1994).

In natural populations, adult size exhibits a huge variability, presumably related to variations in feeding and thermal conditions (Atkinson, 1979; David et al, 1980, 1983; Coyne and Beecham, 1987; Imasheva et al, 1994; Partridge et al, 1994; Moreteau et al, 1995). This phenotypic plasticity cannot be considered as completely neutral. For example, a positive phenotypic correlation exists between size and fitness in nature (Boulétreau, 1978; Partridge et al, 1987). Moreover, Coyne and Beecham (1987) demonstrated that size variations were to some extent heritable in spite of a large environmental component due to plasticity. However, a positive phenotypic correlation between body size and adult fitness components, together with the existence of additive genetic variance for body size, does not necessarily lead to the conclusion that body size is the target of selection (Rausher, 1992).

Up to now, quantitative genetic variations among natural populations, including latitudinal clines, have generally been investigated at a single temperature (with the exception of Coyne and Beecham, 1987), most often 25 °C (David et al, 1983; David and Capy, 1988; Capy et al, 1993). On the other hand, natural selection, which is presumed to be responsible for the clines, acts at various temperatures in different localities and, in all cases, upon highly variable phenotypes. Moreover, temperature is the most important abiotic factor explaining geographic distribution and abundance of species in *Drosophila* (David et al, 1983; Parsons, 1983; Hoffmann and Parsons, 1991). Thus, for a better understanding of these problems, several temperatures must be investigated and compared. In other words, we have to investigate the relationship between developmental temperature and phenotypes, ie, the reaction norms of various traits.

Generally, authors who were interested in the genetics and evolution of reaction norms only considered two environments and consequently linear norms (Via and Lande, 1985, 1987; Scheiner and Lyman, 1989, 1991; De Jong, 1990; Scheiner, 1993a; Via, 1993). Gavrillets and Scheiner (1993) underlined, however, the necessity of studying nonlinear norms and proposed to model them using polynomial adjustments. Indeed, when a broad range of environments (eg, temperature) is investigated, norms of quantitative traits are as a rule nonlinear (David et al, 1983, 1990, 1994; Delpuech et al, 1995).

A recent controversy has developed concerning the genetics of plasticity. Various authors have considered that the mean value of a trait and the shape of the reaction norm should be distinguished. In other words, genes regulating the position of the curve (trait mean value genes) and genes regulating plasticity (shape genes) might coexist (Bradshaw, 1965; Scheiner and Lyman, 1989, 1991; Scheiner et al, 1991; Weber and Scheiner, 1992; Scheiner, 1993ab; Gavrillets and Scheiner, 1993). But this conception was criticized by Via (1993, 1994) who considered it an unnecessary complication, and recent papers have tried to reconcile these two approaches (Van Tienderen and Koelewijn, 1994; Via et al, 1995).

Analysing plasticity leads to several related questions. What is the genetic basis of the reaction norms, and are there specific genes for their shape? What is the

significance of the norm? Is it a consequence of internal constraints or is it adaptive, ie, shaped by natural selection?

It is generally recognized that, before developing a theory on the evolution of reaction norms, many more empirical data are needed, relating the norms with ecological adaptations and life history parameters. In this respect, it will be easier to compare different species (Harvey and Pagel, 1991) since a larger evolutionary time should have permitted a broader divergence of the norms, especially if they were shaped by natural selection. In this paper, we investigated the reaction norms of size traits of a natural population of *D simulans* from France, and compared the results with those obtained for the sibling *D melanogaster* from the same locality (David et al, 1994). We found similarities between the two species but, more interestingly, numerous significant differences. These differences demonstrate that, within a relatively short evolutionary time (about 2 million years) reaction norms have diverged. The possible adaptive significance of these variations is discussed.

MATERIALS AND METHODS

A *D simulans* population was collected in a vineyard in Pont de la Maye near Bordeaux (southern France). Variability of size according to temperature was analyzed, and compared to a population of *D melanogaster* collected in the same locality and previously studied (David et al, 1994).

The isofemale lines method was used. Wild living females were collected with banana traps and used to establish 20 isofemale lines, and ten of them were then randomly chosen. For each, ten pairs of the first laboratory generation were used as parents. They oviposited at room temperature (20 ± 2 °C) for about half a day. A rich feeding medium, based on killed yeast, was used for the development (David and Clavel, 1965). Such a food prevents crowding effects which could affect fly size. Density ranged between 100 and 200 eggs per vial. Vials with eggs were then transferred to one of seven experimental constant temperatures (12, 14, 17, 21, 25, 28, 31 °C). Measured flies thus correspond to the second laboratory generation. Such a procedure is a necessity for obtaining enough offspring (see Moreteau et al, 1995 for discussion). It also eliminates possible maternal effects and provides Hardy-Weinberg proportions within lines.

From each line at each temperature, ten females and ten males were randomly taken. Their wing and thorax lengths were measured with a micrometer in a binocular microscope. Total wing length was measured from the articulation on the side of the thorax to the distal tip. Thorax was measured on a left side view, from the base of the neck to the tip of the scutellum. Analyses were made directly on measurements expressed in $\text{mm} \times 100$, since a preliminary analysis with log-transformed data failed to show any scaling effect.

Statistical analyses and orthogonal polynomial adjustments were made with STATISTICA software (Statistica Statsoft Inc, 1993).

RESULTS

Variation of wing and thorax length: mean of the ten lines

Reaction norms

The response curves (fig 1) show that females are larger than males in both species and that *D melanogaster* is larger than *D simulans*. In both species, a maximum seems to exist at a low temperature. A steep decrease from this maximum is observed when temperature increases, and a shorter one when temperature decreases. In both species, significant differences exist between the reaction norms of wing and thorax. Finally *D simulans* seems to exhibit its maxima for both traits at lower temperatures than *D melanogaster*. This problem will be analyzed further.

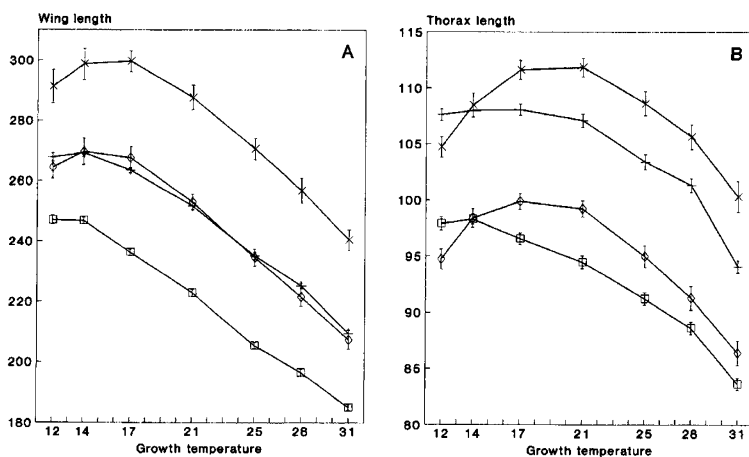


Fig 1. Variations of wing (A) and thorax (B) length according to growth temperature in both species. Vertical bars indicate the confidence interval of the mean (2 se). *D melanogaster*: females (×), males (◊). *D simulans*: females (+), males (□).

Sources of variation

Variations were investigated simultaneously on the two traits in *D simulans* with MANOVA (table I). Sex and temperature are the main sources of variation. A highly significant line effect demonstrates their genetic heterogeneity. The temperature–line interaction, also highly significant, shows that the reaction norms of the different lines are not parallel but exhibit different shapes. Finally the sex–temperature interaction means that males do not react exactly as the females do. These results are similar to those obtained in *D melanogaster* (David et al, 1994), except that the sex–line interaction, which is not significant in *D simulans*, was significant in *D melanogaster*.

Table I. Results of MANOVA on wing and thorax length in *D simulans*.

Source of variation	df	Wilk's λ	F	
Sex	2, 8	0.001 35	2 966	***
Temperature	12, 106	0.001 63	210.3	***
Line	18, 2 518	0.728	24.02	***
Sex-temperature	12, 106	0.239	9.245	***
Sex-line	18, 2 518	0.980	1.410	NS
Temperature-line	108, 2 518	0.710	4.353	***
Sex-temperature-line	108, 2 518	0.903	1.218	NS

df: degree of freedom of numerator and denominator; F: variance ratio; significance: NS non-significant; *** $P < 0.001$.

Correlation between sexes and sexual dimorphism

Male-female correlations were analyzed considering the mean values of each line (table II). There was no temperature effect on the coefficients of correlation (ANOVA, not shown). Average correlation is significantly lower for wing in *D simulans* (0.66 ± 0.07 versus 0.91 ± 0.05 in *D melanogaster*), but similar for thorax in both species (0.71 ± 0.06 and 0.76 ± 0.16).

Table II. Pearson's coefficients of correlation between male and female values of the ten isofemale lines in *D simulans*.

	12 °C	14 °C	17 °C	21 °C	25 °C	28 °C	31 °C	Mean
Wing	0.52	0.93	0.47	0.81	0.77	0.68	0.42	0.66 ± 0.07
Thorax	0.46	0.67	0.59	0.93	0.86	0.83	0.63	0.71 ± 0.06
Ratio	0.65	0.59	0.85	0.82	0.91	0.55	0.40	0.68 ± 0.07

In *D melanogaster*, mean values were: 0.91 ± 0.05 for the wing, 0.76 ± 0.16 for the thorax, 0.87 ± 0.05 for the ratio.

Sexual dimorphism was calculated at each temperature and for each line as the female/male ratio, and submitted to ANOVA (not shown). For wing and thorax, only the temperature effect was significant while the line effect was also highly significant in *D melanogaster*. A nested ANOVA including the two species (not shown) demonstrated highly significant species differences. The two traits (wing and thorax) provide the same information. In the two species, the two sexes are more similar when reared at low temperature (temperature effect). The female/male ratio of *D simulans* is characterized by lower values than in *D melanogaster* (species effect, see David et al, 1994) and by a decrease between 28 and 31 °C (temperature-species interaction).

Covariation between wing and thorax; the wing/thorax ratio**Wing–thorax correlation**

The wing–thorax correlation was investigated at the individual (= within lines) and at the line (= between line means) levels (table III). At the individual level, the values did not vary significantly with temperature; the average phenotypic correlations were 0.71 for females and 0.77 for males and were similar to those obtained in *D melanogaster* (David et al, 1994). For the lines, average values were superior in males (0.79 versus 0.66) but not significantly so (*t* test, not shown). In *D melanogaster*, values were quite similar: 0.73 in males and 0.78 in females.

Table III. Coefficients of correlation between wing and thorax lengths at various temperatures in *D simulans*.

Temperature (°C)	Within lines		Between lines	
	Females	Males	Females	Males
12	0.76 ± 0.05	0.76 ± 0.03	0.47	0.85
14	0.78 ± 0.06	0.77 ± 0.02	0.42	0.60
17	0.72 ± 0.06	0.79 ± 0.04	0.53	0.60
21	0.61 ± 0.11	0.72 ± 0.08	0.84	0.86
25	0.76 ± 0.07	0.77 ± 0.03	0.82	0.91
28	0.67 ± 0.06	0.82 ± 0.04	0.83	0.83
31	0.70 ± 0.08	0.76 ± 0.04	0.71	0.90
Mean	0.71 ± 0.03	0.77 ± 0.02	0.66 ± 0.07	0.79 ± 0.05

Within-lines correlations were calculated at the individual level and averaged over lines (mean ± se). Between-lines correlations were calculated between the means of ten lines.

Wing/thorax ratio

Average curves (fig 2) have a general decreasing sigmoid shape in the two species, but values are much lower in *D simulans*.

Statistical analyses (ANOVA, not shown) demonstrated highly significant effects of temperature (which explains 87% of total variation) and lines. Two-factor interactions were significant as was the triple-factor one. Similar conclusions were obtained in *D melanogaster* (David et al, 1994). On the other hand, the sex effect was not significant, and sexual dimorphism was very reduced for the ratio in both species (see fig 2).

Phenotypic and genetic variability**Within-line variability**

For easier comparison between characters, a relative measure was used: the coefficient of variation (CV) (see David et al, 1994). A major difference between the

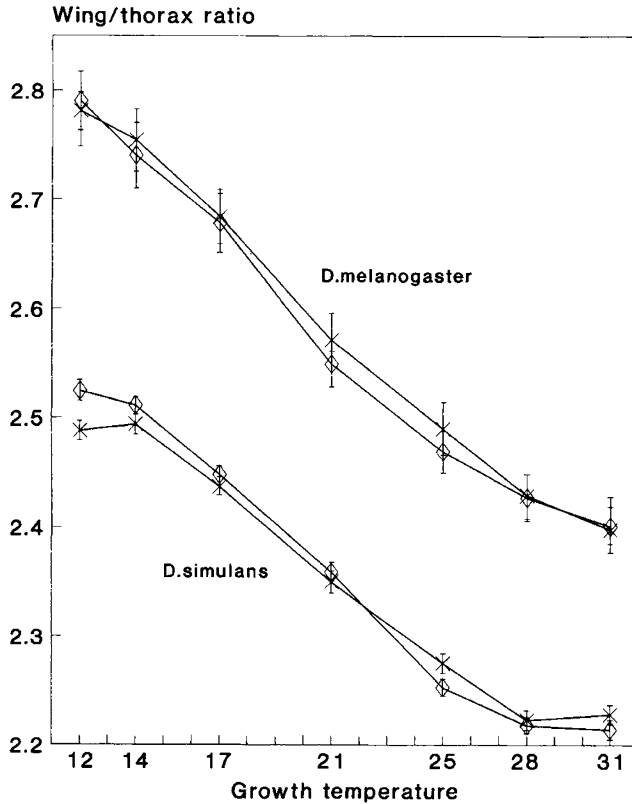


Fig 2. Variations of wing/thorax ratio according to growth temperature in both species. Vertical bars indicate the confidence interval. Females (×); males (◇).

two species concerned the levels of variability. Values were higher in *D. simulans* at high temperatures for the wing (25–31 °C) and the wing/thorax ratio (21–31 °C), and for the thorax over the whole temperature range. Mean values for the seven temperatures are, respectively for wing, thorax, and wing/thorax ratio 2.16 ± 0.18, 2.40 ± 0.21, 1.58 ± 0.15 in *D. simulans*, and 1.97 ± 0.17, 1.96 ± 0.21, 1.40 ± 0.15 in *D. melanogaster*.

Between-line variability

The between-line variance was analyzed by calculating the coefficient of intraclass correlation t , for each sex at each temperature, which is an indicator of isofemale line heritability (Hoffmann and Parsons, 1988). Values of t for wing and thorax are given in table IV. For wing length, a marked species effect is observed, with very different overall means: 0.14 ± 0.03 for females and 0.22 ± 0.05 for males in *D. simulans*, versus 0.58 ± 0.03 and 0.51 ± 0.03 in *D. melanogaster*. For thorax length, values are more similar: 0.25 ± 0.06 (females) and 0.30 ± 0.05 (males) in *D. simulans* versus 0.37 ± 0.04 and 0.30 ± 0.04 in *D. melanogaster*.

Table IV. Coefficients of intraclass correlation of *D simulans*.

	12 °C	14 °C	17 °C	21 °C	25 °C	28 °C	31 °C	Mean
Wing								
Females	0.09	0.06	0.09	0.19	0.30	0.19	0.06	0.14 ± 0.03
Males	0.23	0.08	0.14	0.44	0.31	0.31	0.05	0.22 ± 0.05
Thorax								
Females	0.00	0.11	0.34	0.39	0.41	0.32	0.19	0.25 ± 0.06
Males	0.21	0.25	0.28	0.46	0.47	0.31	0.13	0.30 ± 0.05
Ratio								
Females	0.21	0.33	0.47	0.18	0.36	0.25	0.20	0.29 ± 0.04
Males	0.12	0.33	0.43	0.33	0.27	0.33	0.04	0.26 ± 0.05

These results are illustrated in figure 3 as a correlation between male and female t values. In *D simulans*, t values for the two traits can be divided into two groups: high values (= higher heritability) are observed at medium temperatures (21, 25, 28 °C) and low values at extreme temperatures (12, 14, 31 °C). Means of these two groups are 0.34 ± 0.03 and 0.12 ± 0.02 respectively and statistically different (Student's test, not shown). In *D melanogaster*, no temperature effect was observed for the wing, but a difference between high and low temperatures was observed for the thorax, with a higher genetic variability at high temperatures.

For the wing/thorax ratio (table IV), the general mean calculated on 14 observations is 0.27 ± 0.03 , much lower than in *D melanogaster* (0.57 ± 0.02).

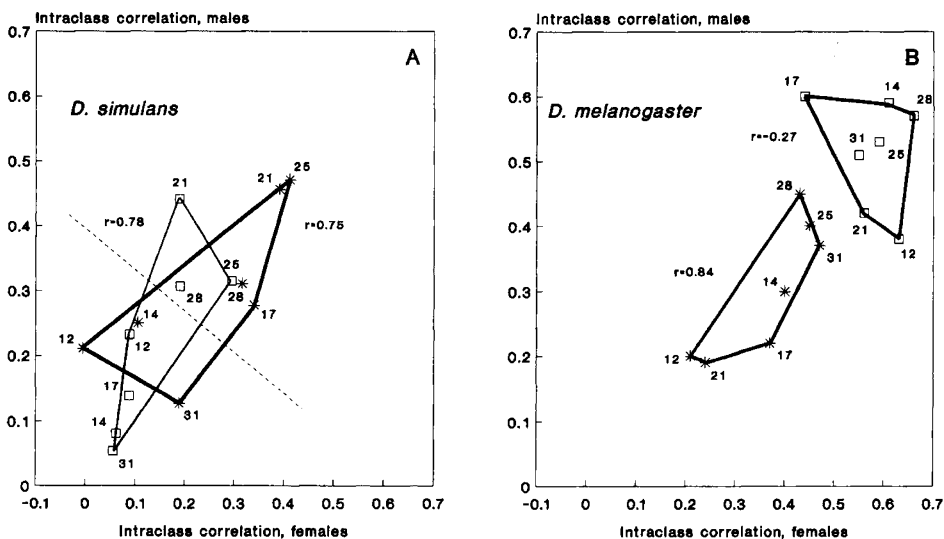


Fig 3. Males plotted against females, intraclass correlations (isofemale heritability) for wing (\square) and thorax ($*$) length at various temperatures. A: *D simulans*; B: *D melanogaster*. The temperature of development is indicated for each point.

Analysis of the shape of reaction norms: slope variations and derivative curves

Wing and thorax

For each isofemale line, length variation for a given temperature interval allows the calculation of a slope (ie, length variation per degree), by a linear intrapolation. Repeating this process for successive intervals produces an empirical derivative of the reaction norm.

An ANOVA (not shown) was conducted on the slopes in *D simulans*. Results were similar for wing and thorax with a very significant temperature effect, demonstrating nonlinear norms. Contrarily to *D melanogaster*, there was no significant sex effect. No line effect was detected, as in the sibling species. In the two species a clear line-temperature interaction shows that derivative curves have different shapes among lines. Finally, a highly significant sex-temperature interaction is present, which was not found in *D melanogaster*.

Average curves and single line curves are given in figure 4, for wing in females only. In the two species, average curves (fig 4a) show a progressive decrease from positive to negative values. These values are significantly lower at low temperature in *D simulans* and not significantly greater than zero. This means that the point where this derivative curve crosses the null line, which corresponds to the temperature of maximum size (TMS), is far less obvious in *D simulans* than in *D melanogaster*, especially for the thorax (see also fig 1). This observation is confirmed by the examination of the curves of different lines (fig 4b). Indeed in *D simulans*, wing length never reached the zero value in two lines, and for thorax length (not shown) the slope often crossed the null line several times. Hence in *D simulans*, a TMS can be calculated by using the average curves, but not for each isofemale line. Average curves point TMS values at 13.5 °C for wing and at 16 °C for thorax in *D simulans*, and at 16 and 19 °C respectively in *D melanogaster*. In other words TMS values appear to be lower in *D simulans* than in *D melanogaster*.

For comparing the two traits, slopes were standardized and expressed as a percentage of the mean (curves not shown). With such a transformation (David et al, 1994), the amplitudes of variation for the two traits become similar. In *D melanogaster* the variation range was greater: the overall phenotypic plasticity seems to be less pronounced in *D simulans*.

Wing/thorax ratio

Slopes of the wing/thorax ratio were calculated in the same way and an ANOVA (not shown) demonstrated a major effect of temperature, a low sex effect, no line effect but a significant line-temperature interaction.

Average slope variations are illustrated in figure 4c for females. In the two species, average derivative curves are U-shaped indicating that the maximum phenotypic plasticity occurs at intermediate temperatures, and also that the wing/thorax ratio varies according to a decreasing sigmoid curve (see fig 2). A regular feature in *D simulans* is that the derivative curve is always above that of *D melanogaster*. Notably,

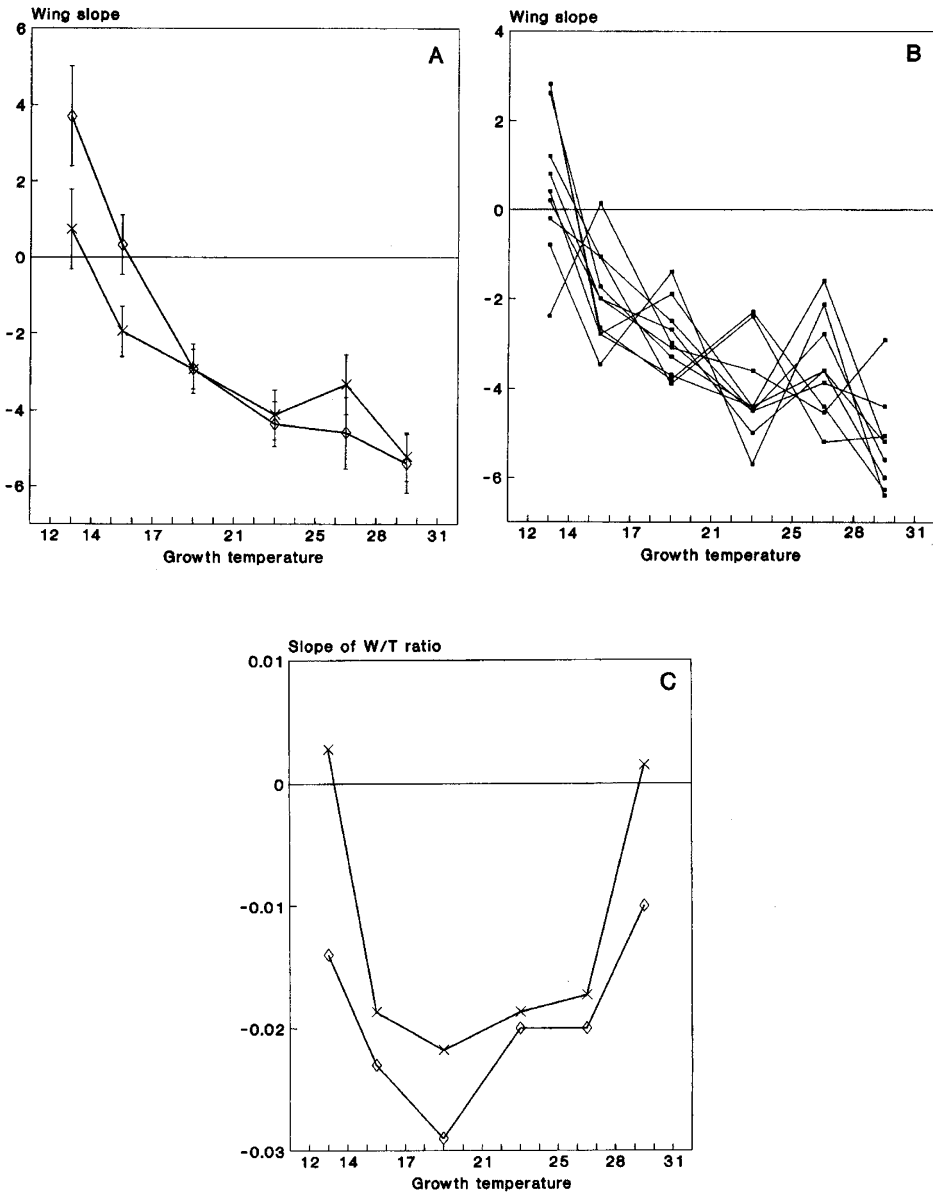


Fig 4. Variations of the slopes of reaction norms of females. **A:** wing average curves in both species; **B:** wing curves of the ten lines in *D. simulans*; **C:** average curves of wing/thorax ratio in both species. For **A** and **B**, zero value slope indicates the TMS. *D. melanogaster* (◇); *D. simulans* (×).

at extreme temperatures, zero values correspond to the fact that the curve of the wing/thorax ratio was horizontal (see fig 2). Moreover, the overall amplitude of variation is larger in *D simulans*.

Analysis of the shape of the reaction norms: polynomial adjustments

Degree of polynomial adjustments

After a theoretical study of linear norms, Gavrillets and Scheiner (1993) suggested that nonlinear norms should be adjusted to second degree polynomials, according to the formula $P(t) = g_0 + g_1t + g_2t^2$ (if we are dealing with temperature, $P(t)$ is the phenotype value at temperature t). The authors proposed for g_0 , the intercept, a genetic significance fixing a basic value to the studied trait, while g_1 , the slope, could be a genetic parameter of adaptation to the environment, and g_2 a genetic parameter of curvature. A second degree polynomial implies that the derivative curve (ie, slope variation) is linear. Such was not the case for the three traits (see fig 4), so that at least a third degree adjustment should be used. Incomplete polynomials could also be used, for instance with no t^2 term. The validity of the various adjustments was assessed by adjusted R^2 values, a poor adjustment being characterized by a low adjusted R^2 . A third degree equation proved to be convenient for the wing/thorax ratio. For wing and thorax lengths, considering the similar shapes in the two species, we imposed a constraint on the adjustment, ie, the existence of a plausible TMS calculated by solving the equation $P'(t) = 0$. For third and fourth degrees, two or three solutions were obtained respectively, which needed to be checked to know which one corresponded to the overall maximum. Finally, for overall homogeneity, all the wing and thorax curves were adjusted to fourth degree polynomials, even those which were compatible with third degree polynomials. Also, similar adjustments were made with the data of *D melanogaster* to compare the two species. Such adjustments were not made in a previous paper (David et al, 1994).

Wing and thorax

Even with fourth degree polynomials, there were still some inadequate TMS values, for instance, 6.3 °C for a male wing. This often occurred from an abnormal value at a single temperature (= rearing accident?) which modified the adjustment equation and thus the TMS. Such cases represented six out of the 40 adjustments made on *D simulans*, but only two of them (for male thorax) deviated from a reasonable value.

Choosing a fourth power polynomial leads to much more heterogeneous g_i parameters than an adjustment in t^2 . For instance, for females wing in *D simulans*, the ten g_0 values ranged from 62 to 69 with the t^2 adjustment, and from -79 to +93 with the t^4 adjustment. A similar conclusion was obtained for all other parameters. Fortunately, calculation of critical points, such as TMS values, provided much less variable values, thus confirming previous observations on ovariole number (Delpuech et al, 1995).

In both sexes of *D simulans* thorax TMS values were generally higher than wing ones, as in *D melanogaster*. Also significantly higher values were demonstrated in

females (ANOVA, not shown). In *D simulans* an overlap of TMSs of the two traits was observed, contrarily to *D melanogaster*. Mean values are given in table V and compared to those of *D melanogaster*. In all cases, TMS values are significantly higher for the latter species. Another striking species difference is the large dispersal among lines of *D simulans* contrasting with a better homogeneity in *D melanogaster* (see CVs in table V). Finally, in all cases, values of males and females of the same line were positively correlated, suggesting that they provide, at least in part, the same genetic information.

Table V. Values and comparison (Student test) of the temperatures of maximum size (TMS) of the two species, for wing and thorax lengths, in both sexes.

	<i>D melanogaster</i>			<i>D simulans</i>			Comparison		
	n	m (°C)	CV	n	m (°C)	CV	t	P	
Wing									
Males	10	14.82	3.8	10	11.62	21.8	3.9	0.0011	**
Females	10	15.59	4.3	10	13.03	10.9	5.2	0.0006	***
Thorax									
Males	10	17.61	4.7	8	14.59	17.6	3.5	0.0028	**
Females	10	19.08	6.3	10	15.88	17.1	3.4	0.0031	**

n: number of considered lines; *m*: mean of the *n* lines TMS; CV: coefficient of variation over the *n* TMS values; *t*: Student's parameter; significance: ** $P < 0.01$; *** $P < 0.001$.

As in David et al (1994), values of both sexes were averaged for each trait. A scatter plot of wing and thorax TMS values (fig 5) clearly contrasted the two species. Interestingly, a positive correlation is found in *D melanogaster* while a non-significant but negative correlation is found for the eight lines of *D simulans* (excluding two lines with aberrant TMS for male thorax). The between-line heterogeneity seems to be mainly due to thoracic variations.

Taking all values into consideration, average curves were also adjusted to the fourth degree and gave TMS values of 13.5 °C (females) and 12.4 °C (males) for the wing, and of 16.1 °C (females) and 13.2 °C (males) for the thorax. These values are lower than in *D melanogaster* (respectively 15.6 and 14.8 °C for wing, 19.2 and 17.6 °C for thorax). They are close to the mean values of the ten lines given in table V and thus characterize the species. Interestingly, the g_i parameters of the average curves were similar to the mean values of the g_i of the ten lines.

Wing/thorax ratio

The g_i parameters of the third degree polynomial were very variable; CVs ranged between 16.5 and 40% for the four female coefficients (mean CV = 28%) and between 22 and 65% (mean CV = 46%) for males. Curves were then characterized by their temperature of inflexion point (TIP), ie, the temperature where the second derivative becomes null.

One line posed a problem in both sexes (aberrant inflexion point value because of a hyperbolic rather than sigmoid shape) and was excluded. TIPs (fig 6) ranged

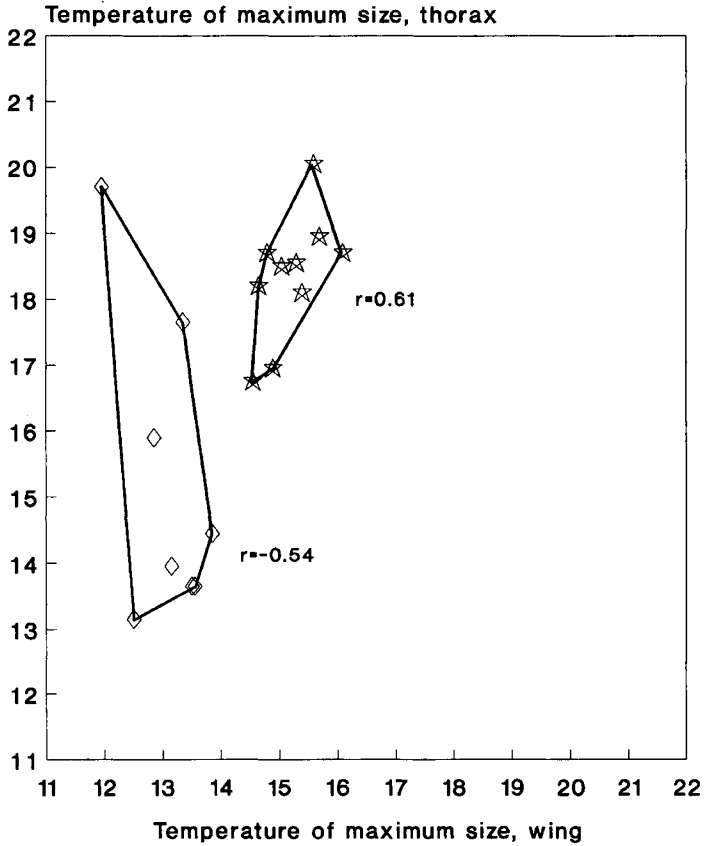


Fig 5. Relationship between TMS of wing and thorax in both species (for each line, both sexes are averaged). In *D simulans*, only eight points are presented because two lines gave aberrant TMS for male thorax. *D melanogaster* (\star); *D simulans* (\diamond).

between 19.9 and 22.8 °C (mean: 21.1 ± 0.3 °C) in females and between 19.9 and 21.3 °C (mean: 20.6 ± 0.2 °C) in males. There was neither line nor sex effect (ANOVA, not shown).

In *D melanogaster*, the same adjustments produced far more variable g_i coefficients: mean CV of 69% in females and 92% in males, ie, more than twice as large as in *D simulans*. This also resulted in a much greater dispersal of the TIP values of the different lines (see fig 6). Also the TIPs were on the average significantly lower (ANOVA, not shown) in *D melanogaster* than in *D simulans*: 19.0 ± 0.9 °C in females and 16.9 ± 1.2 °C in males.

A final observation was that for a given temperature, the ratio of the polynomially adjusted wing value to the polynomially adjusted thorax value was the same as the polynomially adjusted wing/thorax ratio.

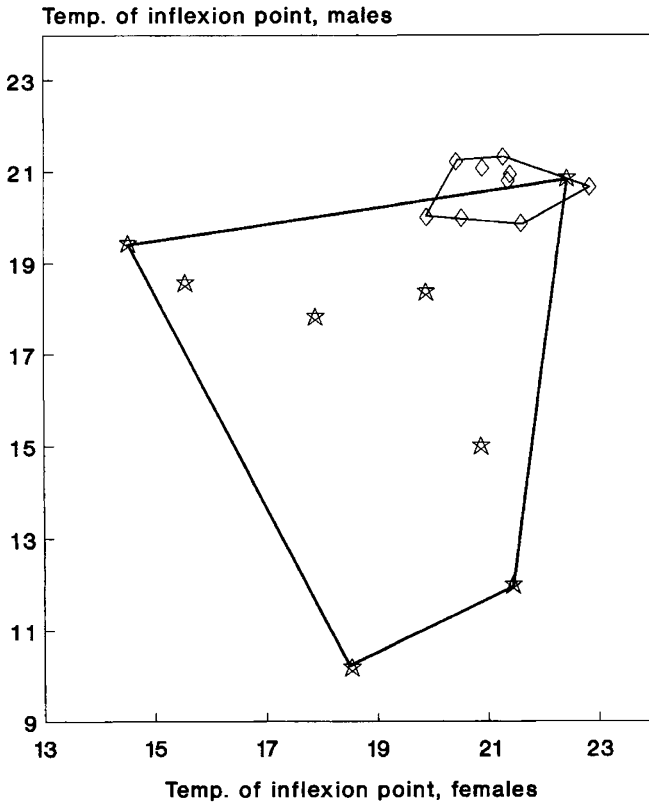


Fig 6. Relationship between temperatures of inflexion point of males and females in both species. Only eight points for *D melanogaster* and nine for *D simulans* are presented because of aberrant values. *D melanogaster* (☆); *D simulans* (◇).

DISCUSSION AND CONCLUSION

Our results need to be discussed from two different points of view: a methodological approach for the description of reaction norms, and the comparative evolutionary biology of the two sibling species. A major, still unsolved problem, will be to decide which species is better adapted to a warmer environment.

How should empirical reaction norms be investigated?

In *Drosophila*, genetic plasticity of quantitative traits such as wing and thorax length was first investigated over two environments (Scheiner and Lyman, 1989, 1991; Scheiner et al, 1991; Weber and Scheiner, 1992; Scheiner, 1993a) and a linear model was used. When a broad range of environmental conditions is used, as such was the case here, most reaction norms are, however, nonlinear (David et al, 1983,

1994; Gavrilets and Scheiner, 1993) and this raises a major problem: what is the best way to describe and analyse the shape of the curve? Factors of variation can be identified with ANOVA or MANOVA, as well as numerous interactions which demonstrate, for example, that the norms significantly differ among isofemale lines from the same population. More precise analyses are however needed for describing the norms, and two kinds of methods may be used: slope variations and mathematical adjustments.

Analysis of slope variations was used by David et al (1990) for demonstrating different pigmentation norms in successive abdominal segments. This method can be of general use for comparing different traits or species, and significant differences may be easily demonstrated. Also the overall shape of the reaction norm may be inferred from the shape of its derivative. In *D simulans*, and contrarily to *D melanogaster* (David et al, 1994), this method was not satisfactory (problems in TMS values determination) and the shapes of the curves had to be studied by mathematical adjustments.

An adjustment to a mathematical model should be a better method but numerous equations could be chosen. In the present case there was no a priori reason for guiding the choice and thus we used a general method, ie, a polynomial adjustment, as suggested by Gavrilets and Scheiner (1993) and Via et al (1995). Because of the great variability among the polynomial coefficients of various lines, it appeared difficult to give them a genetic sense, contrarily to what has been suggested (Gavrilets and Scheiner, 1993). These parameters are, however, conveniently used for calculating critical points of the curves, especially the temperature of maximum size (TMS) for wing and thorax lengths or the temperature of inflexion point (TIP) for the wing/thorax ratio. Reaction norms appear to be better characterized by these points, which are less variable and seem to have a biological significance, and presumably also a genetic basis. In this respect, we found that TMS values of males and females of the same line were positively correlated in both species and, among lines, thorax and wing TMS values were also correlated in *D melanogaster* (David et al, 1994). Interestingly in *D melanogaster*, calculating the TMS values either by considering slope variations or with polynomial adjustments provided similar results. In *D simulans*, fourth power polynomials had to be used instead of quadratic ones for a better characterization of TMS values. But even in that case, the adjustment could not be performed for some isofemale lines. This may reflect either true genetic peculiarities of these lines or some experimental imprecisions. This problem needs further investigation, for example, by analyzing the same line over two successive generations.

Similarities between the two species

Similarities between closely related species are expected because of phylogenetic constraints and also from a possible similarity of their ecological niches (Harvey and Pagel, 1991). In the present study, numerous similarities were observed, which are briefly summarized below.

In the two species females are larger than males, and this could be a general result in most *Drosophila*. The female/male ratio gives similar data for wing and thorax and could be considered as a good measure of sexual dimorphism. This dimorphism

is a phenotypically plastic trait with minimum values at low temperatures in both species.

Reaction norms of the three characters (wing and thorax length and wing/thorax ratio) are nonlinear and present the same sources of variation. Wing and thorax both exhibit a maximum at low temperature. The response of the wing/thorax ratio to temperature is a sigmoid decreasing curve, similar for both sexes.

In all cases, coefficients of intraclass correlation (t) were significantly greater than zero, demonstrating (Hoffmann and Parsons, 1988) a high heritability of the traits. Moreover a regular line-temperature interaction indicates significant genetic variations in the shapes of reaction norms among isofemale lines.

The within-line CVs varied with temperature in all cases, with maxima at extreme temperatures. This is likely due to an increase of the developmental noise under stressful conditions. In both species, the wing/thorax ratio is less variable (lower CVs) than the traits themselves. This is due to the fact that wing and thorax variations are correlated at the individual level.

Differences between the two sibling species

Numerous and important differences were found between the two species. These differences demonstrate that canalization during development is not very strong so that the investigated traits could diverge, either as a consequence of drift or of ecological adaptation.

As already known from numerous observations (see Capy et al, 1993) *D simulans* is a smaller species. We may argue that speciation was accompanied by size gene variations, determining the position of the reaction norms on the Y axis.

Sexual dimorphism presented different reaction norms in the two species. It is unfortunate that we do not have a convenient evolutionary theory for sexual dimorphism in organisms like *Drosophila* (Charnov, 1993).

Heritability of size traits was different in the two species, contrarily to what was found by Capy et al (1994) in a broad survey of numerous populations reared at a single temperature (25 °C). In our study of two sympatric populations, *D melanogaster* appeared on the average more variable than *D simulans*. In both species variations of isofemale line heritabilities were observed according to developmental temperature, but with different patterns for different traits. These differences are difficult to interpret, and many more comparative studies should be undertaken.

At the within-line level, phenotypic variability exhibits a major environmental component (Falconer, 1989) and thus reflects in some way the reactivity of individuals to minor variations in culture vials (eg, food desiccation or larval competition). This reactivity may be estimated by considering the CVs. *D simulans* appeared more variable than *D melanogaster* for thorax length over the whole temperature range and for the other two traits at high temperatures only. These results are somewhat surprising, because phenotypic variability was previously found to be similar in the two species (Capy et al, 1994). A problem remains: are these results general to the species or specific to the studied populations?

A major difference between the two species concerns their TMS values, which are much lower in *D simulans* than in *D melanogaster*, with a translation toward

the left in *D simulans*. As the thermal ranges are about the same in the two species (Cohet et al, 1980, and this work), it was more difficult to calculate TMS values in *D simulans*. A careful analysis showed that, besides the translation, the shapes of the norms were somewhat different in the two species. Within species, a significant line-temperature interaction demonstrates genetic variations in the curve shapes. Finally, the heterogeneity of TMS values between lines is larger in *D simulans* than in *D melanogaster*, in spite of a lower genetic variability within each temperature in the former species. These observations argue in favor of a genetic regulation of the reaction norm shape.

A last but major difference between the two species concerns the wing/thorax ratio which is much smaller in *D simulans* and presents higher TIPs.

All these differences support a general trend: the more the two species are compared, the more they appear different (see Capy et al, 1993, 1994 for discussion and references).

Reaction norms and the thermal adaptation of the two species

Since we investigated the effects of developmental temperature, we must ask the question: is one species better adapted to a colder or warmer climate? Answering this question is difficult, since we have conflicting observations.

Although the thermal laboratory ranges are similar (12–31 °C) in the two species (Cohet et al, 1980), ecological surveys (Louis, 1983) have shown that *D simulans* is generally more abundant than *D melanogaster* in warm temperate and subtropical regions, while it is rare or even absent in cold regions where *D melanogaster* is still present. These observations lead to the classical interpretation that *D simulans* is less tolerant to cold than *D melanogaster* (Parsons, 1983). So our results are surprising. Indeed, even if the biological meaning of a TMS is not clearly established, we expect that a maximum should be related to some optimum (Parker and Maynard-Smith, 1990; Gabriel and Lynch, 1992; Stearns, 1992). Could we suppose, then, that *D simulans* is more adapted to cold than *D melanogaster*, contrarily to what was believed up to now, and that reaction norms indicate the direction of adaptation? In fact, this hypothesis is not unlikely. Indeed, from an ecological point of view, *D melanogaster* enters human buildings where it is protected during winter, whereas this is not the case for *D simulans* (Rouault and David, 1982). So the latter will suffer lower temperatures than *D melanogaster* during winter, and hence will be selected for cold tolerance. Two other arguments support this hypothesis. Firstly, in *D melanogaster*, males reared at 12 or 13 °C are sterile, whereas this is not the case in *D simulans* (David, unpublished observations). Secondly, in competition experiments at 25 °C, *D melanogaster* generally eliminates *D simulans*, while the reverse occurs at temperatures below 20 °C (Tantawy and Soliman, 1967; Montchamp-Moreau, 1983).

Other observations suggest however a reverse interpretation. The wing/thorax ratio, which is inversely proportional to wing loading and wing beat frequency (Pétavy et al, 1992, 1996) decreases with temperature, presumably in relation with a better muscular efficiency at higher temperature (Reed et al, 1942). In other words, a low wing/thorax ratio could indicate a warm adapted phenotype, and according to this hypothesis, *D simulans* would be adapted to a warmer climate than

D. melanogaster. Moreover the TIP, which corresponds to a maximum of plasticity, is higher in *D. simulans*. Even if the possible relationship between the TIP and the optimum flight temperature remains to be investigated, this could support the hypothesis of a better adaptation of *D. simulans* to a warmer environment.

Molecular studies at the within-population level have shown that *D. simulans* was generally more polymorphic than *D. melanogaster* (Aquadro et al, 1988; Begun and Aquadro, 1991; Aquadro, 1992). To explain this observation, the former authors suggested that the population effective number is higher in *D. simulans*, due to a higher migration rate and a better dispersal capacity. In this respect the lower wing/thorax ratio in *D. simulans* could be more a dispersal adaptation than a thermal adaptation. However, in spite of numerous studies (Brodsky, 1994) we do not know what is the best strategy for dispersal, ie, high speed correlated with high wing loading and relatively short flight duration, or vice versa.

In conclusion, the two sibling species which are increasingly investigated as a model for evolutionary studies, appear very different when more thoroughly analyzed, and interpretations are difficult. Concerning the evolution of reaction norms and their possible relationship with thermal adaptation, further comparative studies are needed, either on geographic populations of the two sibling species and on other *Drosophila* species clearly adapted to warm or cold climates.

ACKNOWLEDGMENT

This work benefited from a grant from the French Ministère de l'Environnement (EGPN committee) to JR David.

REFERENCES

- Aquadro CF (1992) Why is the genome variable? Insights from *Drosophila*. *Trends Genet* 8, 355-362
- Aquadro CF, Lado KM, Noon WA (1988) The rosy region of *Drosophila melanogaster* and *Drosophila simulans*. I. Contrasting levels of naturally occurring DNA restriction map variation and divergence. *Genetics* 119, 875-888
- Ashburner M (1989) *Drosophila: A Laboratory Manual*. Cold Spring Harbor Laboratory Press
- Atkinson WD (1979) A field investigation of larval competition in domestic *Drosophila*. *J Anim Ecol* 48, 91-102
- Begun DJ, Aquadro CF (1991) Molecular population genetics of the distal portion of the X chromosome in *Drosophila*: evidence for genetic hitchhiking of the *yellow-achaete* region. *Genetics* 129, 1147-1158
- Boulétreau J (1978) Ovarian activity and reproductive potential in a natural population of *Drosophila melanogaster*. *Œcologia (Berl)* 35, 319-342
- Brodsky AK (1994) *The Evolution of Insect Flight*. Oxford Univ Press, Oxford
- Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. *Adv Genet* 13, 115-155
- Capy P, Pla E, David JR (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

- Capy P, Pla E, David JR (1994) Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans*. II. Within-population variability. *Genet Sel Evol* 26, 15-28
- Cavicchi SD, Guerra G, Giorgi G, 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
- Charnov EL (1993) *Life History Invariants*. Oxford Univ Press, Oxford
- Cohet Y, Voudibio J, David JR (1980) Thermal tolerance and geographic distribution: a comparison of cosmopolitan and tropical endemic *Drosophila* species. *J Therm Biol* 5, 69-74
- Coyne JA, Beecham E (1987) Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. *Genetics* 117, 727-737
- David JR, Clavel MF (1965) Interaction entre le génotype et le milieu d'élevage. Conséquences sur les caractéristiques du développement de la drosophile. *Bull Biol Fr Belg* 99, 369-378
- David JR, Capy P (1988) Genetic variation of *Drosophila melanogaster* natural populations. *Trends Genet* 4, 106-111
- David JR, Cohet Y, Fouillet P, Arens MF (1980) Phenotypic variability of wild collected *Drosophila*: an approach toward understanding selective pressures in natural populations. *Egypt J Genet Cytol* 9, 51-66
- David JR, Allemand R, Van Herrewege J, Cohet Y (1983) Ecophysiology: abiotic factors. In: *The Genetics and Biology of Drosophila* (M Ashburner, HL Carson, JN Thompson, eds), Academic Press, London, Vol 3D, 105-170
- David JR, Capy P, Gauthier JP (1990) Abdominal pigmentation and growth temperature in *Drosophila melanogaster*: similarities and differences in the norms of reaction of successive segments. *J Evol Biol* 3, 429-445
- David JR, Moreteau B, Gauthier JP, Pétavy G, Stockel J, Imasheva AG (1994) Reaction norms of size characters in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis. *Genet Sel Evol* 26, 229-251
- De Jong G (1990) Genotype-by-environment interaction and the genetic covariance between environments: multilocus genetics. *Genetica* 81, 171-177
- Delpuech JM, Moreteau B, Chiche J, Pla E, Voudibio J, David JR (1995) Phenotypic plasticity and reaction norms in temperate and tropical populations of *Drosophila melanogaster*: ovarian size and developmental temperature. *Evolution* 49, 670-675
- Falconer DS (1989) *Introduction of Quantitative Genetics*, third edition, Longman, New York
- Gabriel W, Lynch M (1992) The selective advantage of reaction norms for environmental tolerance. *J Evol Biol* 5, 41-59
- Gavrilets S, Scheiner SM (1993) The genetics of phenotypic plasticity. V. Evolution of reaction norm shape. *J Evol Biol* 6, 31-48
- Harvey P, Pagel MD (1991) *The Comparative Method in Evolutionary Biology*. Oxford Univ Press, Oxford
- Hoffmann AA, Parsons PA (1988) The analysis of quantitative variation in natural populations with isofemale strains. *Genet Sel Evol* 20, 87-98
- Hoffmann AA, Parsons PA (1991) *Evolutionary Genetics and Environmental Stress*. Oxford Univ Press, Oxford
- Imasheva AG, Bubli OA, Lazebny OE (1994) Variation in wing length in Eurasian natural populations of *Drosophila melanogaster*. *Heredity* 72, 508-514
- Louis J (1983) Les espèces dominantes de drosophiles dans les peuplements de l'Europe atlantique et méditerranéenne. *Ann Soc Entomol Fr* 19, 167-173

- Montchamp-Moreau C (1983) Interspecific competition between *Drosophila melanogaster* and *Drosophila simulans*: temperature effect on competitive ability and fitness components. *Genet Sel Evol* 15, 367-378
- Moreteau B, Capy P, Alonso-Moraga A, Munoz-Serrano A, Stockel J, David JR (1995) Genetic characterization of geographic populations using morphometrical traits in *Drosophila melanogaster*: isogroups versus isofemale lines. *Genetica* 96, 207-215
- Parker GA, Maynard-Smith J (1990) Optimality theory in evolutionary biology. *Nature* 348, 27-33
- Parsons PA (1983) *The Evolutionary Biology of Colonizing Species*. Cambridge Univ Press, Cambridge
- Partridge L, Hoffman A, Jones JS (1987) Male size and mating success in *Drosophila melanogaster* and *D pseudoobscura* under field conditions. *Anim Behav* 35, 468-476
- Partridge L, Barrie B, Fowler K, French V (1994) Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* 48, 1269-1276
- Pétavy G, Gauthier JP, David JR (1992) Plasticité phénotypique et normes de réaction de la taille chez *Drosophila melanogaster*: influence de la température de développement. *Bull Soc Ecophysiol* 17, 107-113
- Pétavy G, David JR, Moreteau B (1996) Les variations induites par la température sur les paramètres de taille et du vol des drosophiles sont-elles adaptatives? *Bull Soc Zool Fr* (in press)
- Powell JR (1974) Temperature-related genetic divergence in *Drosophila* size. *J Hered* 65, 257-258
- Rausher MD (1992) The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* 46, 616-626
- Reed SC, Williams CM, Chadwick LE (1942) Frequency of wing-beat as a character for separating species, races and geographic varieties of *Drosophila*. *Genetics* 27, 349-361
- Reiss MJ (1989) *The Allometry of Growth and Reproduction*. Cambridge Univ Press, Cambridge
- Rouault J, David JR (1982) Evolutionary biology of *Drosophila melanogaster* and *D simulans*: a behavioral divergence in microhabitat selection. *Acta Oecol Gener* 3, 331-338
- Scheiner SM (1993a) Genetics and evolution of phenotypic plasticity. *Ann Rev Ecol Syst* 24, 35-68
- Scheiner SM (1993b) Plasticity as a selectable trait: reply to Via. *Am Nat* 142, 371-373
- Scheiner SM, Lyman RF (1989) The genetics of phenotypic plasticity. I. Heritability. *J Evol Biol* 2, 95-107
- Scheiner SM, Lyman RF (1991) The genetics of phenotypic plasticity. II. Response to selection. *J Evol Biol* 4, 23-50
- Scheiner SM, Caplan RL, Lyman RF (1991) The genetics of phenotypic plasticity. III. Genetic correlations and fluctuating asymmetries. *J Evol Biol* 4, 51-68
- STATISTICA Statsoft Inc (1993) *Statistica, release 4.5*. Tulsa, OK, USA
- Stearns SC (1992) *The Evolution of Life Histories*. Oxford Univ Press, Oxford
- Tantawy AO, Soliman MH (1967) Studies on natural populations of *Drosophila*. VI. Competition between *D melanogaster* and *D simulans*. *Evolution* 21, 34-40
- Van Tienderen PH, Koelewijn HP (1994) Selection on reaction norms, genetic correlations and constraints. *Genet Res* 64, 115-125
- Via S (1993) Adaptive phenotypic plasticity: target or by-product of selection in a variable environment? *Am Nat* 142, 352-365
- Via S (1994) The evolution of phenotypic plasticity: what do we really know? In: *Ecological Genetics* (LA Real, ed), Princeton University Press, Chichester, 35-57

- Via S, Lande R (1985) Genotype–environment interaction and the evolution of phenotypic plasticity. *Evolution* 39, 505-522
- Via S, Lande R (1987) Evolution of genetic variability in a spatially heterogeneous environment: effects of genotype–environment interaction. *Genet Res* 49, 147-156
- Via S, Gomulkiewicz R, De Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH (1995) Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol Evol* 10, 212-217
- Weber SL, Scheiner SM (1992) The genetics of phenotypic plasticity. IV. Chromosomal localization. *J Evol Biol* 5, 109-120