Phenotypic plasticity of body size in *Drosophila*: effects of a daily periodicity of growth temperature in two sibling species

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Abstract. Variation of wing and thorax length under thermoperiodic growth conditions was analysed in four strains of two sibling species, *Drosophila melanogaster* and *D. simulans*, from two European localities. Results were compared to those obtained with constant temperatures ranging from 12 to 31 °C.

Under constant temperatures the data basically confirmed previous results: concave reaction norms for wing and thorax length; a monotonically decreasing norm for wing : thorax ratio; and an increasing norm for sex dimorphism (female : male ratio). Phenotypic variability was maximum at extreme temperatures and minimum at middle ones. Slight differences were observed according to the geographical origin: the difference between strains from Bordeaux (France) and Cordoba (Spain) was maximum at low temperatures but disappeared at about 28 °C.

According to the temperatures chosen, alternating thermal regimens had either no effect or produced a significant size reduction, probably reflecting a periodic stress. The magnitude of this effect was proportional to the amplitude of the thermoperiod but not to the quality (cold or heat) of the stress. In a similar way, the wing : thorax ratio was either not modified or reduced significantly, indicating that wing length was relatively more affected than thorax length by alternating thermal regimens. Sex dimorphism also showed either no change or a significant increase, indicating that males were relatively more reactive than females to alternating conditions. Finally, regimens of broad amplitudes increased the phenotypic variability, again an indication of stressful effects. All these observations should be taken into account when analysing phenotypic variability in nature and trying to understand natural selection in wild-living populations.

Key words. Cold and heat stress, *Drosophila melanogaster*, *D. simulans*, norms of reaction, phenotypic variability, sex dimorphism, thorax and wing length.

Introduction

Temperature is a most important environmental factor for ectotherms and its selective role may be analysed in numerous ways (Precht *et al*., 1973; Cossins & Bowler, 1987; Leather, 1988; Leather *et al*., 1993; Klingenberg & Spence, 1997). A fairly general observation concerns a genetic increase of body size, often observed in relation to latitude (Atkinson, 1994). This rule applies to several *Drosophila* species with a broad geographical range, in which latitudinal clines of size have been found (Capy *et al*., 1993; Karan *et al*., 1998, 1999a). In all these studies, genetically determined body size is strongly correlated to latitude, which is itself highly correlated with average annual temperature. This observation explains the widespread interpretation according to which average temperature is responsible for body size variation, even if the selective mechanisms are not obvious (McCabe & Partridge, 1997). We must also consider that clines are generally

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analysed on laboratory flies, grown at a single constant temperature under good feeding conditions, to minimize environmental variability and maximize genetic effects. Natural selection, responsible for clines is, however, acting on natural populations which are submitted, often simultaneously, to a diversity of selective pressures. With respect to size, it is known that wild-living flies are smaller and much more variable than laboratory-grown flies of similar genotypes (David *et al.*, 1970; Moreteau *et al.*, 1995; David *et al.*, 1997; Gibert *et al.*, 1998), but there are only a few papers that have compared the size of wild flies from different latitudes (Coyne & Beecham, 1987; Robertson, 1987; Imasheva *et al.*, 1994; James *et al.*, 1997). Moreover, *Drosophila* species exhibit several generations a year, which are submitted to different temperatures in different seasons. The phenotypic changes related to developmental temperature are now well documented by laboratory studies in several species (David *et al.*, 1994, 1997; Moreteau *et al.*, 1997; Morin *et al.*, 1997; Karan *et al.*, 1999b), but the analysis of seasonal phenotypic variation in nature remains an almost unexplored field. Finally, in a given place and a given season, environmental temperature is not a constant but exhibits a daily cycle with sometimes a broad amplitude. After pioneer research on the effects of alternating temperatures on survival, fecundity and rate of development (see Siddiqui & Barlow, 1972, for *Drosophila*), tolerance to a daily thermal stress is a new area of research investigated in a few species of ectotherms (Loeschcke *et al.*, 1997).

In the present paper, the question was asked: are there some specific effects of alternating thermal regimens upon body size? Results of course must be compared with data obtained under constant temperature, and producing the same average growth rate. The definition of an equivalent developmental temperature (Pétavy *et al.*, 2001) provides such a comparative means with more ease in this case than the commonly used 'degree day' measures. Four mass populations were analysed of two sympatric species, *D. melanogaster* and *D. simulans*, from two European localities, Bordeaux in south-western France and Cordoba in southern Spain (see Discussion). These two temperate places exhibit quite different climates. A diversity of specific effects of periodic temperatures was found, especially when the daily amplitude exceeded 10 °C. Diurnal temperature variations must be taken into account when trying to explain phenotypic variations in nature.

Materials and Methods

Flies of both species were collected in autumn 1993 near Bordeaux, France and near Cordoba, Spain. Mass cultures of the four strains were initiated with at least 30 isofemale lines and kept in culture bottles without selection at 21 °C under LD 16 : 8 h. Adults of these strains were transferred to egg collection chambers with a yeasted plate of agar medium. One hour later, they were given a new plate and the first set of eggs discarded. Three hours later, the newly laid eggs were examined under the microscope and the normal-looking ones distributed in groups of 80 in culture vials containing a high

nutrient, killed yeast food (David, 1962), so that crowding effects were minimal. Vials were transferred to incubators regulated at $\pm$ 0.2 °C, either at a constant or an alternating thermal regimen with two daily 12-h cycles, described by two values, the first one being the lower phase, the other the higher one. In all cases the duration of development was measured. For each alternating regimen, we calculated an equivalent developmental temperature, that is the constant temperature which would provide the same duration (see Pétavy *et al.*, 2001, for details). In each experiment, the four strains were simultaneously investigated at a given constant temperature and under an alternating regimen with an equivalent developmental temperature close to the constant temperature. In all cases, embryonic development began at the mildest phase of the alternating regimen.

A few hours after emergence, adults were transferred to vials containing fresh food and kept at 20–23 °C under natural photoperiod. Four to six days later, the thorax and the left wing were measured on 50 flies of each sex according to David *et al.* (1994) with an accuracy of 5 μm (thorax) or 10 μm (wing).

Besides wing and thorax length of both sexes, two other traits were calculated: the wing : thorax ratio, which is negatively correlated to wing loading and is related to flight capacity (Pétavy *et al.*, 1997), and the sex dimorphism, calculated as a female : male ratio.

Under constant regimens, the response curves, or reaction norms, were adjusted to polynomials (see David *et al.*, 1997). Data obtained under alternating conditions were analysed by considering the differences between the observed values and the reaction norm at the same equivalent developmental temperature.

Variation of size traits in each 50-fly sample was expressed by the coefficient of variation, which avoids scaling effects (David *et al.*, 1978). Between-sample variation was analysed by a *t*-test in simple comparisons, and by a two-way ANOVA in other cases when interactions were investigated.

Results

Reaction norms of wing and thorax length under constant regimens

Eight temperatures were used in both species: 12, 14, 16, 18, 21, 26, 28 and 30 °C. The temperature of 31 °C being almost completely lethal for *D. simulans* (Pétavy *et al.*, 2001), it was used only for *D. melanogaster*.

The corresponding response curves, or reaction norms, are shown in Figs 1 and 2 (open symbols linked by lines). They are nonlinear asymmetric concave curves with maxima below 18 °C. For each species, data were analysed by ANOVA and all effects were significant (Table 1). The strain effect corresponds to a smaller average size of the Spanish flies. But the main conclusion is that the strain × temperature interaction is highly significant in all cases, meaning that the reaction norms are not parallel. More precisely, the Bordeaux flies of both species were significantly bigger than the Cordoba ones for
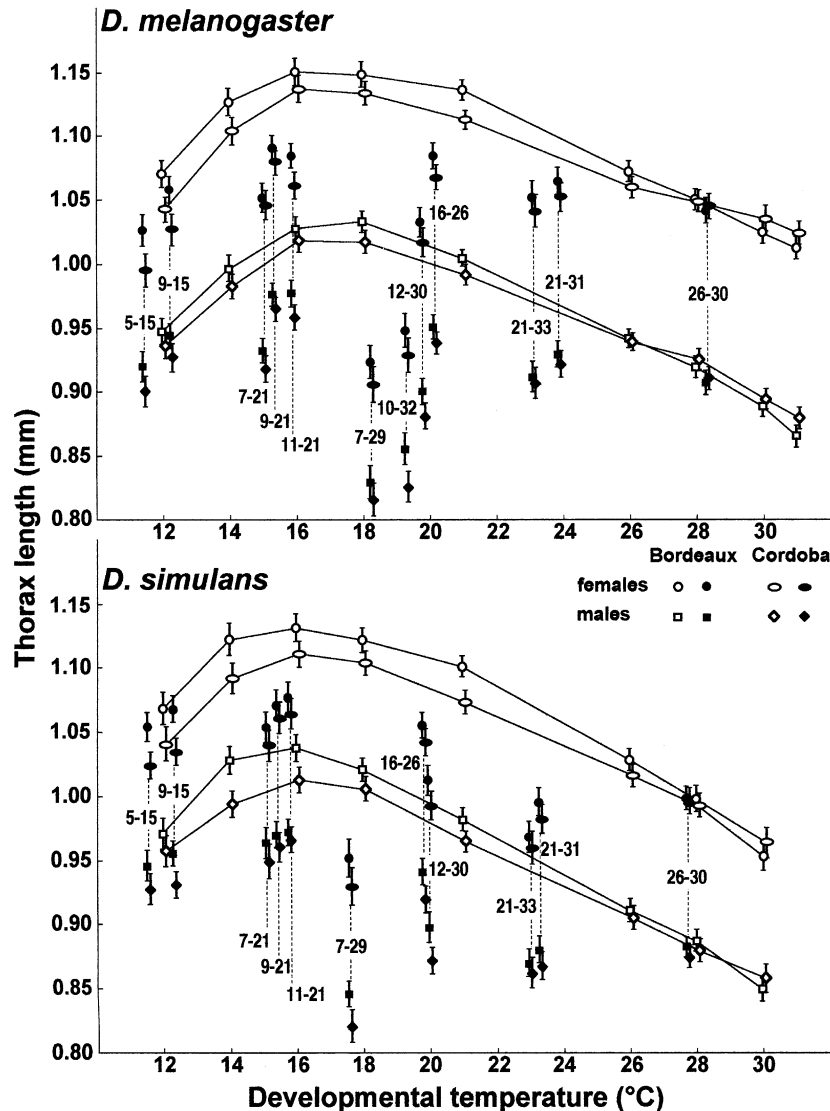
Fig. 1. Mean reaction norms of thorax length at constant temperatures (open symbols linked by lines) and mean values at alternating thermal regimens (closed symbols). Sample size: 50 flies at each temperature. Vertical bars: 95% confidence intervals. For the alternating thermal regimens, growth temperatures are calculated as the equivalent developmental temperatures, which provide the same duration under constant conditions.

both traits investigated at temperatures below 26 °C (see Figs 1 and 2). Above 26 °C, the difference between strains progressively disappeared and even the flies from Cordoba became significantly larger than those from Bordeaux at the most extreme temperature, 31 °C in *D. melanogaster*. Because of their asymmetry, the reaction norms were adjusted to polynomials of degree 3, allowing the calculation of characteristic values, that is a maximum value and the temperature of this maximum (see David *et al.*, 1997). Values, given in Table 2, show that temperatures of maximum values were lower for wing than for thorax length, and lower in *D. simulans* than in *D. melanogaster*, confirming previous data (Morin *et al.*, 1996). There was, however, no consistent difference

according to the geographical origin. For maximum values, those of Cordoba were always lower than those of Bordeaux in both species and both sexes.

We also used the polynomial adjustments to calculate for each sex and species the temperatures at which reaction norms of the two strains intercross, that is, the temperatures of equal values: each value was calculated by equating the expression of the two polynomials. Another means was to equate the expression of regression lines from data obtained between 21 and 30 or 31 °C. In all cases, temperatures of equal values were slightly higher in *D. simulans* than in *D. melanogaster*, the difference being less for thorax length: about 1.4 °C vs. 2 °C for wing length (Table 2).
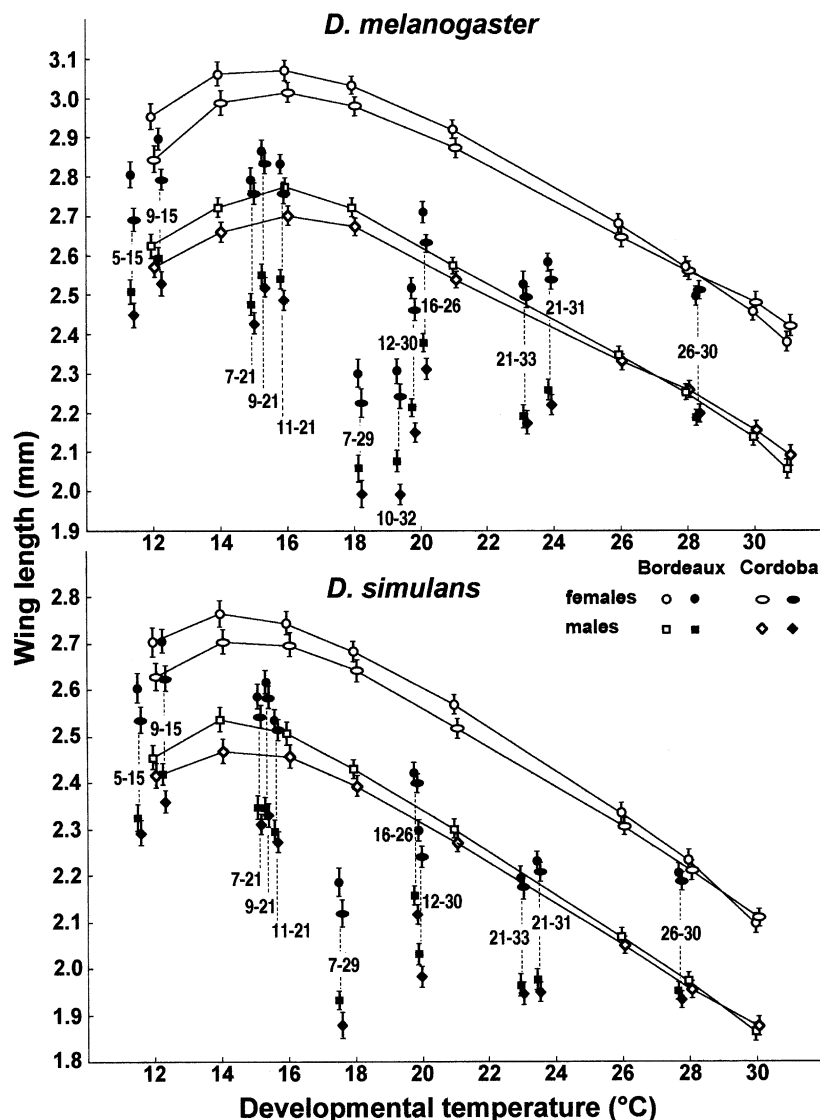
Fig. 2. Mean reaction norms of wing length and average values at alternating thermal regimens. Symbols as in Fig. 1.

Finally, the two species were compared by calculating, for each temperature and each strain, a *D. melanogaster* : *D. simulans* ratio. For wing length, this ratio was greater than unity and increased regularly from 1.06–1.09 at 12 °C to 1.15–1.17 at 30 °C. The ratio was lower for thorax length, increasing from 0.97–1.00 at 12 °C to 1.03–1.07 at 30 °C.

Wing and thorax length under alternating conditions

We used 12 regimens with different amplitudes and mid-temperatures (Table 3). One extreme regimen, 10–32 °C, was almost completely lethal for *D. simulans*. The equivalent developmental temperatures, given in Table 3, are used in the graphs plotting size against growth temperature (closed symbols in Figs 1 and 2).

The major conclusion is that in most cases, adults developed under alternating conditions were significantly smaller than adults grown under constant temperatures and showing the same duration of development. In a few cases, i.e. at 9–15 or 26–30 °C, sizes were identical to those obtained at 12 and 28 °C, respectively. In some other cases, and especially with the extreme 7–29 °C in *D. melanogaster*, adults were significantly smaller than the smallest ones obtained under constant conditions. This may be interpreted by considering that development under alternating regimens of broad amplitude, 22 °C in that case, is a major stress, which does not prevent metamorphosis and adult survival but strongly impairs normal development.

Table 1. Results of ANOVA on thorax and wing length for flies grown at constant temperatures. d.f. = degree of freedom; MS = mean square; F = variance ratio; ***$P < 0.001$; *$P < 0.05$.

			Females		Males	
	Source of variation	d.f.	MS	F	MS	F
Thorax length						
Drosophila melanogaster	Strain	1	0.024	30.7 ***	0.004	6.3 *
	Temperature	8	0.237	306.4 ***	0.313	490.0 ***
	Strain × temperature	8	0.005	6.8 ***	0.003	4.3 ***
	Residual	882	0.001		0.001	
Drosophila simulans	Strain	1	0.055	60.8 ***	0.038	49.9 ***
	Temperature	7	0.363	402.5 ***	0.420	549.9 ***
	Strain × temperature	7	0.005	4.9 ***	0.004	5.2 ***
	Residual	784	0.001		0.001	
Wing length						
Drosophila melanogaster	Strain	1	0.284	64.8 ***	0.152	49.4 ***
	Temperature	8	6.324	1441.6 ***	6.481	2099.1 ***
	Strain × temperature	8	0.053	12.1 ***	0.036	11.5 ***
	Residual	882	0.004		0.003	
Drosophila simulans	Strain	1	0.288	78.9 ***	0.190	63.6 ***
	Temperature	7	6.058	1659.7 ***	6.322	2113.7 ***
	Strain × temperature	7	0.018	4.8 ***	0.003	4.3 ***
	Residual	784	0.004		0.003	

Table 2. Characteristics of mean reaction norms for thorax and wing length at constant growth temperatures. The temperature of maximum value was calculated from polynomial adjustments of degree 3 on average data. The temperature of equal value for the Bordeaux and Cordoba strains was calculated either from the polynomial equation (first value) or from a linear regression on data obtained between 21 and 30 or 31 °C (second value).

		Maximum value (mm)		Temperature of maximum value (°C)		Temperature of equal value (°C)	
		Thorax length	Wing length	Thorax length	Wing length	Thorax length	Wing length
D. melanogaster							
Females	Bordeaux	1.153	3.061	17.8	15.9		
	Cordoba	1.136	3.003	17.9	16.4		
						28.7–28.0	28.6–27.9
Males	Bordeaux	1.030	2.743	17.8	15.9		
	Cordoba	1.016	2.683	17.9	16.1		
						26.7–26.4	27.3–27.0
D. simulans							
Females	Bordeaux	1.130	2.745	17.1	14.4		
	Cordoba	1.108	2.692	17.1	15.0		
						28.8–28.8	29.3–29.6
Males	Bordeaux	1.031	2.510	16.4	14.4		
	Cordoba	1.008	2.460	16.5	14.5		
						28.6–28.8	29.9–30.0

The relationship between thermal amplitude and size variations was analysed by calculating the difference between the actual mean size of adults developed under a given alternating regimen and the theoretical size of the reaction norm at the same equivalent developmental temperature (we considered the norm as linear between the data obtained at the two constant temperatures flanking the equivalent developmental temperature). Because the reaction norms are themselves variable

Table 3. Equivalent developmental temperatures for each alternating thermal regimen (order: increasing mid-temperatures). B^x and C^a = Bordeaux and Cordoba strains.

Alternating temperature regimens (°C)	Thermal amplitude (°C)	Mid-temperature (°C)	Equivalent developmental temperatures			
			D. melanogaster		*D. simulans*	
			B^x	C^a	B^x	C^a
5–15	10	10	11.3	11.4	11.5	11.6
9–15	6	12	12.2	12.1	12.3	12.3
7–21	14	14	15.0	15.0	15.1	15.1
9–21	12	15	15.3	15.3	15.4	15.4
11–21	10	16	15.9	15.9	15.6	15.6
7–29	22	18	18.1	18.2	17.5	17.6
10–32	22	21	19.3	19.3	–	–
12–30	18	21	19.7	19.7	20.0	19.9
16–26	10	21	20.1	20.1	19.8	19.7
21–31	10	26	23.9	24.0	23.5	23.5
21–33	12	27	23.1	23.3	23.0	23.0
26–30	4	28	28.3	28.3	27.7	27.7
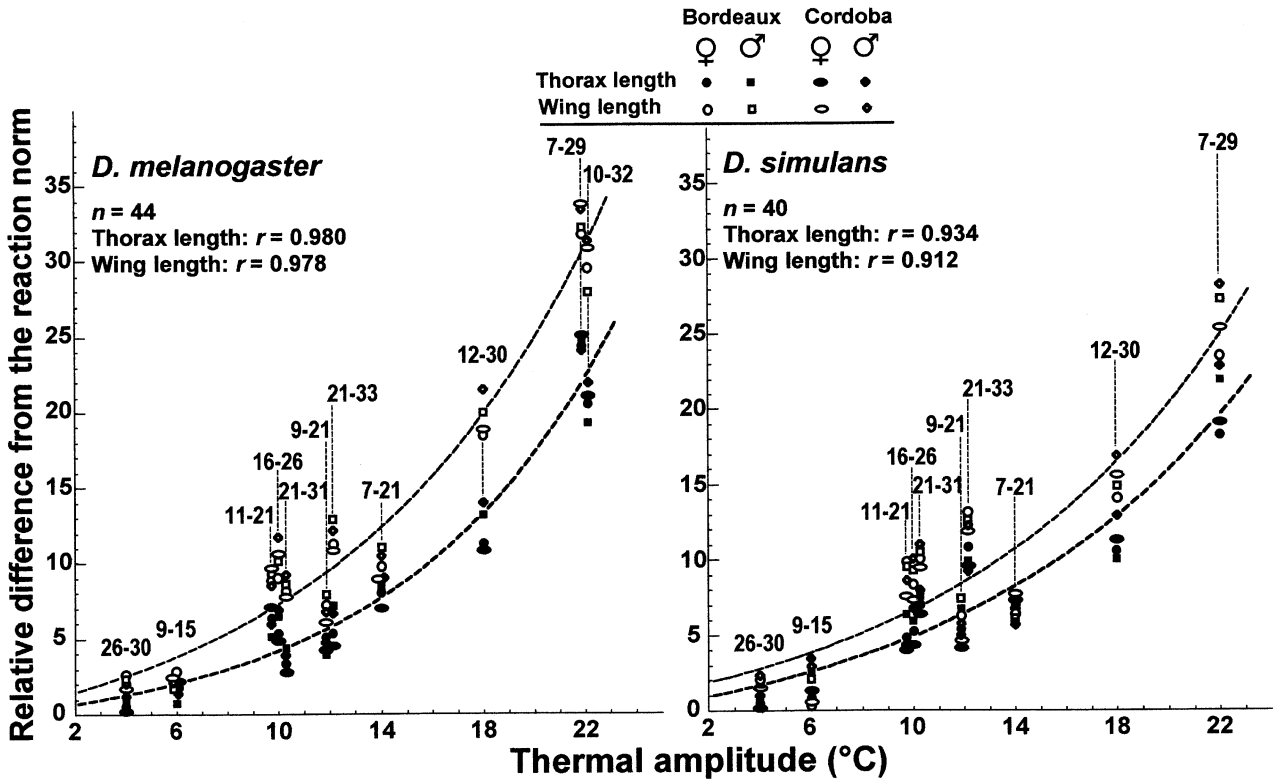
Fig. 3. Relationship between the relative difference with the mean reaction norm (thorax: closed symbols; wing length: open symbols) and the thermal amplitude of each alternating thermal regimen. Vertical scale: relative differences are multiplied by 100. Each dashed curve was calculated from a curvilinear regression following an exponential equation: $D = b + e^{c + kA}$, with D: average relative difference; A: thermal amplitude; e: base of natural logarithms; b, c and k: parameters; n: number of means (both sexes were pooled). Values of k (with D multiplied by 100) for thorax length: 0.118 in *D. melanogaster* and 0.088 in *D. simulans* – for wing length: 0.097 and 0.091, respectively. Each correlation coefficient r was computed with the least squares method.

according to sex, strain and trait, a relative difference was calculated, dividing the absolute difference by the corresponding value of the reaction norm. Figure 3 plots these relative differences as a function of thermal amplitude. For both traits in the two species, a highly positive correlation was observed between the two variables. A more precise analysis showed that a better adjustment was obtained with a curvilinear regression, as shown in Fig. 3 with an exponential model. These adjustments suggest two conclusions: first, the reactivity of wing length to alternating regimens is more pronounced than that of thorax length; second, the reactivity to thermal amplitude is stronger in *D. melanogaster* than in *D. simulans*. Moreover, the proposed model allows a prediction of the average value for a given alternating regimen, knowing the mean reaction norm, the thermal amplitude, the equivalent developmental temperature, and the parameters of the exponential curve.

A further analysis of the data revealed that the relative distance from reaction norms was higher for both traits in the two sexes and species of Bordeaux compared to Cordoba strains under three regimens: 7–21, 9–21 and 21–33 °C (and 11–21 °C in *D. simulans* only). The reverse was observed at regimens of a broader thermal amplitude, i.e. 7–29, 12–30 °C and 10–32 °C in *D. melanogaster*.

Phenotypic variability under constant and alternating conditions

Phenotypic variation of different traits and species were compared by considering the coefficient of variation, which eliminates scaling effects. Data obtained under constant regimens in each species were submitted to ANOVA (not shown). A highly significant effect of developmental temperature was found but no effect of trait, sex or strain. As shown in Fig. 4, the temperature effect corresponded to a minimum coefficient of variation (about 2%) around 21 °C and maximum values were found at both ends of the thermal range (values close to or higher than 3%). These results show that individual variation increases under stressful conditions.

Under alternating regimens, coefficients of variation were either similar or significantly superior to those observed under constant regimens (see Fig. 4). Indeed, for broad thermal amplitudes (7–29, 21–33 and 10–32 °C), average values were higher than 3.5%, and sometimes (7–29 °C) superior to 4% in both species. These observations suggest that alternating conditions may be more stressful than any extreme constant temperature.

Wing : thorax ratio under constant and alternating regimens

The wing length : thorax length ratio is known to be a character of its own, showing a monotonically decreasing reaction norm with increasing growth temperature (David *et al.*, 1994; Morin *et al.*, 1996, 1999). Moreover, it is negatively correlated to wing loading and wingbeat frequency (Pétavy *et al.*, 1997).

Under constant conditions we obtained almost linear decreasing reaction norms, similar between sexes and strains
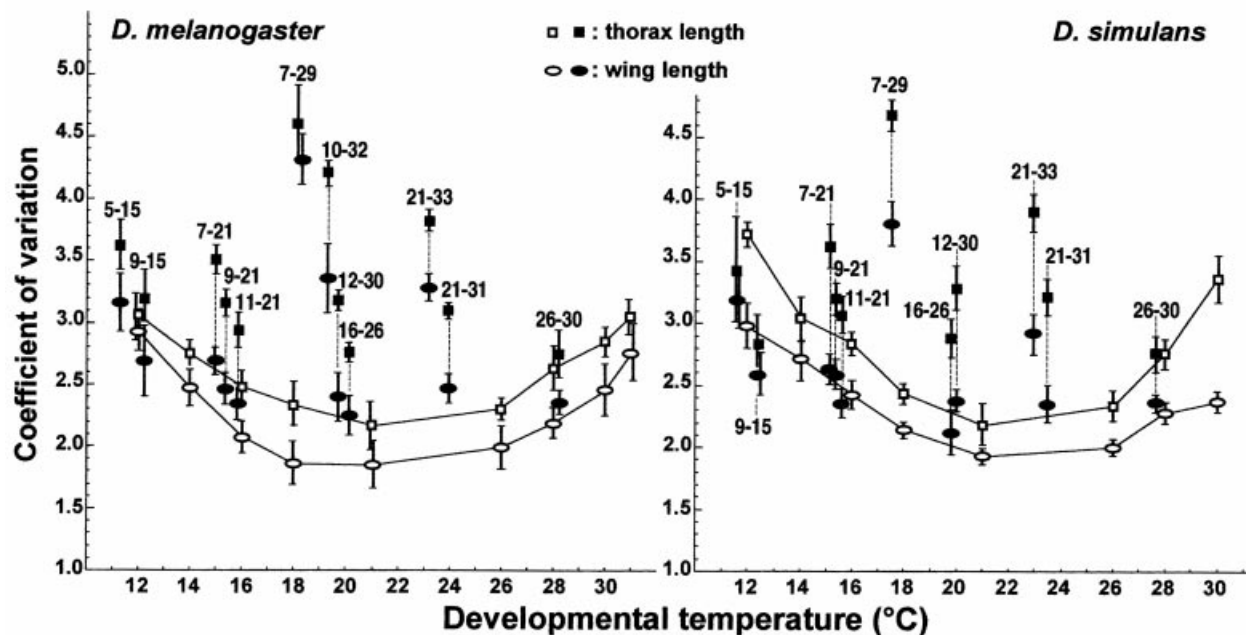
Fig. 4. Coefficients of variation (percentages) for thorax and wing length at constant temperatures (open symbols linked by lines) and alternating thermal regimens (closed symbols). Vertical bars: 95% confidence intervals.
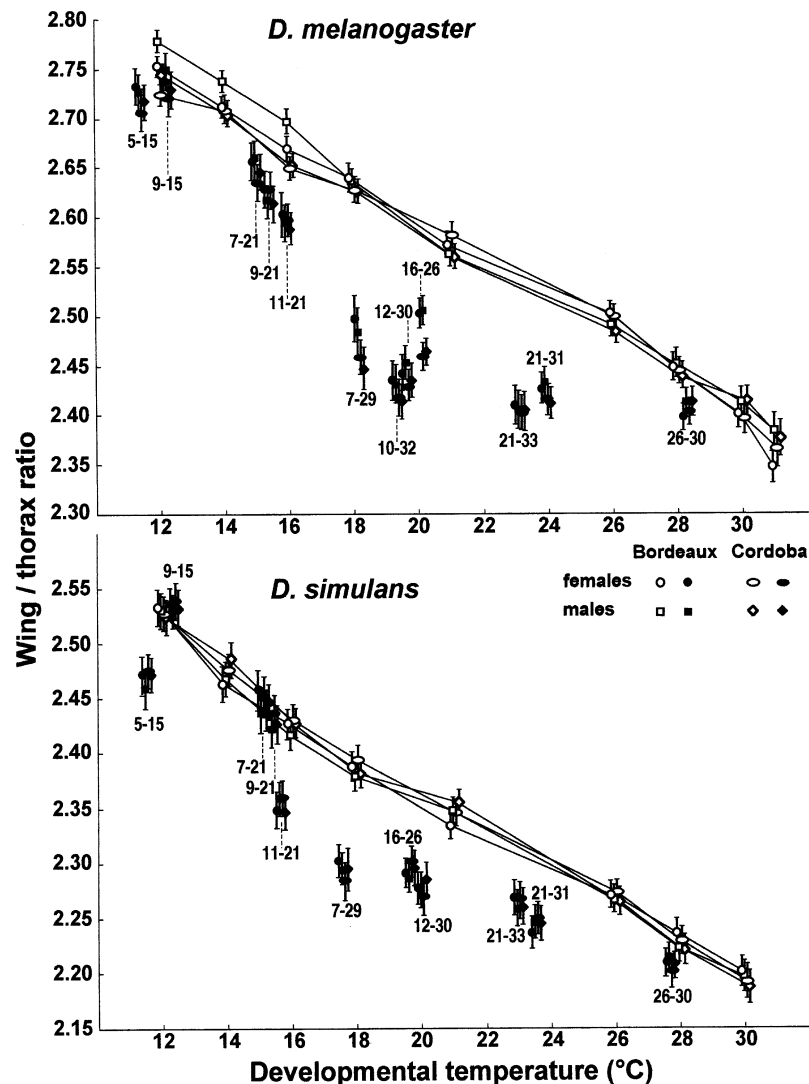
Fig. 5. Reaction norms of the wing : thorax ratio (open symbols linked by lines) and values at alternating thermal regimens (closed symbols). Symbols as in Fig. 1.

of the same species (Fig. 5). As expected, values were much lower in *D. simulans*.

Under alternating regimens, wing : thorax ratios were generally lower than under constant conditions, and the differences were greater with broad thermal amplitudes. Such a decrease means that wing length was relatively more affected than thorax length by the stressful effects of alternating regimens (see also Fig. 3).

Sex dimorphism under constant and alternating conditions

Sex dimorphism, measured as a ratio of female : male value, is known to be a plastic character depending on growth temperature (David *et al.*, 1994; Morin *et al.*,

1999). More precisely the female : male ratio of wing or thorax length increases with developmental temperature. The data under constant conditions confirmed this phenomenon (see Fig. 6) and revealed a significant difference between species, dimorphism being lower in *D. simulans*.

Under alternating regimens, we found values either similar to those obtained under constant conditions or significantly higher. The major differences were sometimes obtained with regimens of broad amplitudes (e.g. 12–30 °C) but not always (see for example the 7–29 and 10–32 °C values in *D. melanogaster*). Moreover, the 26–30 °C regimen, which for length values failed to reveal any specific effect, produced significantly higher sex dimorphism. Clearly the female : male ratio provides specific information. A higher sex dimorphism means that males
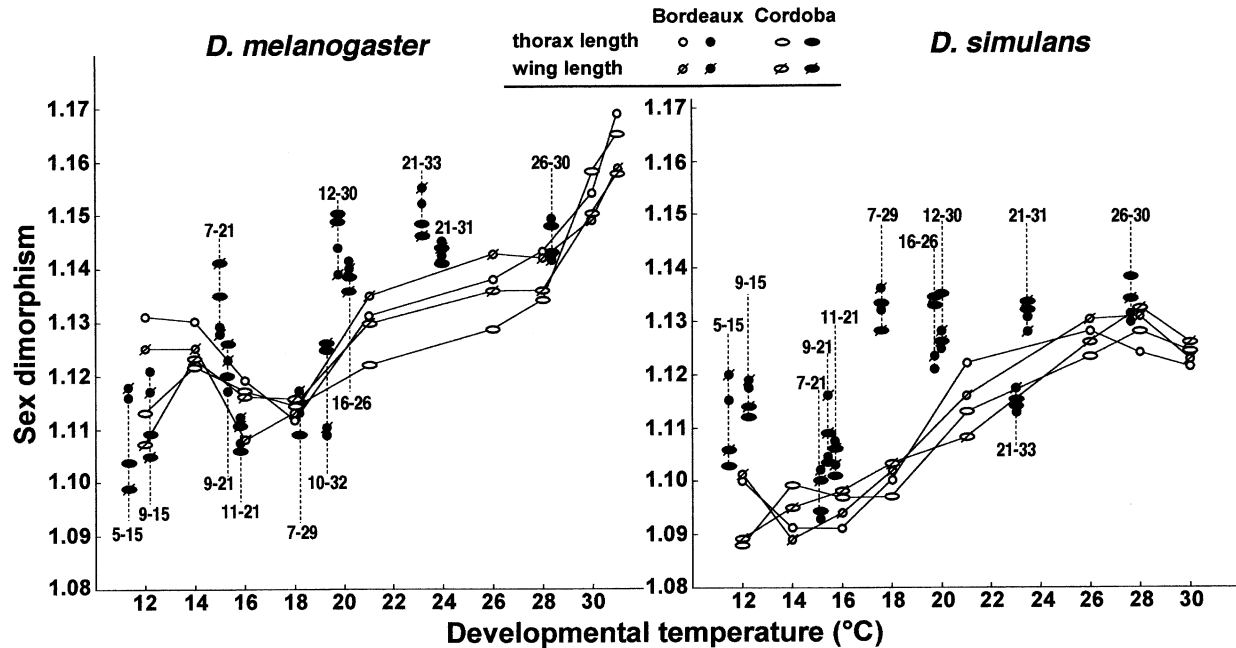
Fig. 6. Sex dimorphism (female : male ratio) for thorax and wing length at constant temperatures (open symbols) and alternating thermal regimens (closed symbols). Symbols as in Fig. 1.

are more affected than females by the stressful conditions of some alternating regimens.

Discussion

The results obtained on four mass laboratory strains under constant conditions are similar to those with isofemale lines from French populations (David *et al.*, 1994; Morin *et al.*, 1996). Wing and thorax lengths exhibit concave reaction norms with maximum values below the optimum (21–22 °C) developmental temperature. The wing : thorax ratio, which is related to wing loading, wingbeat frequency and flight capacity (Pétavy *et al.*, 1997), has a monotonically decreasing norm, whereas sex dimorphism expressed as the female : male ratio increases with growth temperature.

Species differences and geographical variation

Significant differences were found between the two sibling species: compared with *D. melanogaster*, *D. simulans* is slightly smaller, has shorter wings, exhibits its maximum size at a lower temperature, has a higher wing loading and a lower sex dimorphism. Both species exhibit latitudinal clines of size, flies being bigger in populations at higher latitudes (Capy *et al.*, 1993). The European populations revealed slight size difference according to geographical origin; that is, an overall smaller size in the Spanish populations of both species. More interestingly, however, the reaction norms were not parallel,

differences being more pronounced at low temperatures, and null at about 28 °C: climatic adaptation, as revealed by genetical clines, might change not only the average size but the shape of the reaction norm. Such an hypothesis has already been proposed for another plastic trait, abdomen pigmentation of females, in two French populations (Gibert *et al.*, 1996). These data point to the danger of considering a single laboratory temperature (generally 25 °C, see Capy *et al.*, 1993) when investigating genetical clines from natural populations. Although being separated by only 7° of latitude, Bordeaux and Cordoba have quite different climates: the first region is under oceanic influence, Mediterranean contrasts prevail in the other one. The difference between average annual temperatures is about 6 °C (Bordeaux: 14.0 °C; Cordoba: 19.8 °C), and the daily thermal amplitude is always lower in Bordeaux (on average 9.3 °C, monthly range 6.5–11.4 °C) than in Cordoba (on average 12.3 °C, range 8.3–15.1 °C). Natural selection is likely to occur mostly at temperatures below the optimum, and the Bordeaux population may be considered better adapted to colder conditions. Indeed, it expresses a bigger size mostly at temperatures below 21 °C.

Effects of alternating temperature on size traits

Daily thermal variations have revealed specific changes in size traits. As stated in a previous paper (Pétavy *et al.*, 2001), development is still possible even when half of each day is submitted to a temperature outside the viability range, that is, below 11 or above 32 °C. For example, adults were obtained

under 5–15 or 21–33 °C regimens. The harmful effects of excessive cold or heat can be overcome by a return to a milder, more normal temperature. In five cases, we used the thermal optimum (21 °C) as a component of the daily cycle, whereas the other phase was either very cold (7, 9 or 11 °C) or very hot (31 or 33 °C): the cultures were submitted each half-day, either to a cold or to a heat stress. Adult phenotypes were different from those obtained under constant conditions with the same equivalent developmental temperature, but the effects were of the same kind, with no apparent specificity due to cold or heat stress. Therefore, the whole data set with alternating regimens can be described in a general way: either the thermal amplitude is small (less than 5 °C) and its effects are negligible as compared with the reaction norm, or the amplitude is broader and the results lie outside the norm.

For wing and thorax length, alternating regimens generally produced smaller adults. Indeed, for some conditions with a broad amplitude and in which a cold phase alternated with a heat phase (e.g. 7–29 or 10–32 °C), flies were significantly smaller than those grown under the most extreme constant temperature (30 or 31 °C). Considering the whole data set, the magnitude of the effect is mostly related (in an exponential way) to the thermal amplitude, whereas the mid-temperature (or the equivalent developmental temperature) is of little importance. Moreover, alternating regimens always reduced the wing : thorax ratio, but a value lower than at the highest constant temperature was never obtained. Such changes of the ratio are due to a higher reactivity of wing length under alternating regimens, as compared to thorax length. The fact that wing is a two-dimensional structure and thorax a tri-dimensional one might explain this result.

Finally, for sex dimorphism, changes were observed but in an opposite direction, because alternating regimens increased the trait, with males being relatively more affected than females.

A major effect of alternating regimens was to increase variation of size traits, a result that agrees with previously published data on the same species (David *et al.*, 1994; Morin *et al.*, 1996; Karan *et al.*, 1999a). This phenomenon is probably due to an increase in the environmental, non-genetic variance: a daily thermal stress seems to increase the developmental instability.

The two sibling species reacted to alternating regimens in similar ways: *D. simulans* appears slightly more tolerant to cold and less tolerant to heat than *D. melanogaster* (Pétavy *et al.*, 2001), and less reactive to stress caused by alternating temperatures. In a few cases, however, discordant results were obtained between the two species, and especially for sex dimorphism: the 7–29 °C regimen had no effect in *D. melanogaster* but significantly increased the dimorphism in *D. simulans*; the 21–33 °C regimen decreased the dimorphism in this species only.

Relevance for clinal studies

Body size in *Drosophila* is generally considered as a fitness related trait, as bigger females produce more eggs (Boulétreau,

1978; McCabe & Partridge, 1997) and larger males have generally a selective advantage (Partridge & Farquhar, 1983; Partridge *et al.*, 1987; Reeve *et al.*, 2000). In this respect, the present data suggest that alternating temperature regimens with a sufficient amplitude reduces the adult fitness. In some other insect species, e.g. locusts, daily thermoperiodic conditions increase the fitness and improve development and reproduction (Parker, 1930; Zitoune, 1973). Clearly, more extensive studies, including adult physiological traits, are needed in *Drosophila* and other insects.

Finally, the present study helps to explain the phenotypic variability in wild-living populations. It is now well established that, at least in *D. melanogaster*, size variation is much greater in nature than in the laboratory (David *et al.*, 1980; Coyne & Beecham, 1987; Imasheva *et al.*, 1994; Moreteau *et al.*, 1995; Gibert *et al.*, 1998). More precisely, the coefficient of variation of wing length of wild-living adults is always around 7%, whereas it is much less in the laboratory, sometimes as low as 2% (see Gibert *et al.*, 1998). Until now, the only factor known to decrease body size, at a constant temperature, was the quality and quantity of larval food (Bakker, 1961). It was thus assumed that the very small flies often observed in nature had been submitted to some kind of larval food restriction (Moreteau *et al.*, 1995; David *et al.*, 1997; James *et al.*, 1997). The experimental results presented here now suggest another possible cause. Under natural conditions, daily thermal amplitudes greater than 10 °C are frequent, and they can be even much more when the larval resources are exposed directly to sunshine for a few hours a day (Feder *et al.*, 1997; Feder & Krebs, 1997; Loeschcke *et al.*, 1997). Thus, a significant increase in phenotypic variability, as well as an overall size reduction, can be due to a thermoperiodic stress and not to a food shortage. Such a result must be taken into account when trying to explain temperature adaptation in *Drosophila*, and especially latitudinal clines of body size.

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