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Body size reaction norms in *Drosophila melanogaster*: temporal stability and genetic architecture in a natural population

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Abstract – A natural population of *Drosophila melanogaster* was sampled twice over a 5-year interval from the same French locality in the same season. Reaction norms of wing and thorax length and wing/thorax ratio, according to growth temperature (12–31 °C) were analysed in ten isofemale lines for each sample. Reaction norms were very similar between years, showing not only a remarkable stability of the average size but also of the reactivity to temperature. Wing and thorax length reaction norms were characterized by the co-ordinates of their maxima (MV = maximum value of character; TMV = temperature of maximum value). The wing/thorax ratio, which exhibited a decreasing sigmoid norm, was characterized by the co-ordinates of the inflexion point. Again, these characteristic values were found to be very similar for samples between years. The results were further analysed by pooling the 20 lines into a single data set. Heritability was significantly variable according to temperature, but in a fairly irregular way with lowest values at extreme temperatures. Genetic variance of the three traits exhibited more regular variation with a minimum at intermediate temperatures and maxima at extreme high or low temperatures. Such was also the case of evolvability, i.e. the genetic coefficient of variation. Heritability and evolvability were found to be slightly but negatively correlated, showing that they provide independent biological information. The temporal stability of a natural population over the years suggests some stabilizing selection for both mean body size and plasticity. For laboratory evolution experiments, the natural origin population might be useful as a genetic control over time. © Inra/Elsevier, Paris

phenotypic plasticity / growth temperature / wing and thorax length / wing/thorax ratio / evolvability

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Résumé – Normes de réaction de la taille corporelle chez *Drosophila melanogaster* : stabilité temporelle et architecture génétique dans une population naturelle. Une population naturelle de *Drosophila melanogaster* a été échantillonnée deux fois à cinq ans d'intervalle, dans la même localité et à la même saison. Les normes de réaction de la longueur de l'aile et du thorax, ainsi que du rapport aile/thorax, ont été analysées en fonction de la température de développement chez dix lignées isofemelles pour chaque échantillon. Les normes de réaction se sont avérées très semblables dans les deux échantillons, montrant ainsi une remarquable stabilité de la taille moyenne et aussi de la réactivité à la température. Les normes de réaction de l'aile et du thorax ont été caractérisées par les coordonnées de leur maximum (MV = valeur maximale du caractère ; TMV = température de la valeur maximale). Le rapport aile/thorax, qui présente une norme décroissante sigmoïde, a été caractérisé par les coordonnées du point d'inflexion. Ces valeurs caractéristiques ont aussi été trouvées très semblables dans les deux échantillons. Les résultats ont été ensuite analysés en réunissant les 20 lignées dans un seul échantillon. L'héritabilité s'est avérée variable en fonction de la température, mais de façon assez irrégulière avec les valeurs les plus basses aux extrêmes. La variance génétique des trois caractères a présenté une variation plus régulière, avec un minimum aux températures moyennes et des maximums aux températures extrêmes. L'évolvabilité estimée par le coefficient de variation génétique, a montré des variations similaires. L'héritabilité et l'évolvabilité se sont avérées légèrement mais négativement corrélées, montrant qu'elles fournissent des informations biologiques différentes. La stabilité temporelle d'une population naturelle au cours des années suggère une sélection stabilisante à la fois pour la taille moyenne et la plasticité. Dans des expériences d'évolution en laboratoire, la population naturelle d'origine pourrait être utilisée en tant que contrôle génétique au cours du temps.

plasticité phénotypique / température de développement / longueur de l'aile et du thorax / rapport aile/thorax / évolvabilité

1. INTRODUCTION

In microevolutionary studies, an interesting approach is to consider the temporal stability of a given population. A persistant stability is often interpreted as a consequence of balancing selection while regular variations according to environmental changes (e.g. season) may also reveal strong selection forces [25, 42, 44]. Long-term irregular or regular trends in the same locality may be due to drift or to some progressive modification of the environment. Since the pioneering works of Dobzhansky on chromosome inversions in *Drosophila pseudoobscura*, all these different patterns of variation have been observed in various *Drosophila* species, but mostly refer to chromosome rearrangements or allozyme frequencies, with in most cases an adaptive interpretation [27].

For quantitative traits, investigations on natural populations have mainly demonstrated spatial genetic variations such as latitudinal clines in various species [2, 4, 14, 23, 25], and temporal variations are less well documented. This seems to be due to several practical difficulties and to the fact that such variations, if any, are likely to be smaller than those observed across long distances. One difficulty is a lack of consensus on how to measure a quantitative trait. For example, wing size is generally estimated as wing length but there are numerous dimensional parts which have been equated to the length. Another difficulty is the sensitivity of quantitative traits to experimental conditions, such as food, temperature and population density. A related problem is a

frequent lack of repeatability and an apparent instability when the same measurement is undertaken several times on the same population [9, 17]. A final problem is the likelihood of genetic drift or conversely of laboratory adaptation when a population is kept for a long time under laboratory conditions. Facing such difficulties, it has sometimes been argued that natural populations of *Drosophila* are too unstable for a convenient analysis of natural selection upon fitness related traits. According to Rose et al. [36], the analysis of evolutionary mechanisms should be simplified in an "experimental wonderland" by controlling in the laboratory one or a few conveniently chosen environmental factors. This approach was already used in population cages of *Drosophila* for analysing, for example, adaptation to different growth temperatures [1, 6, 33]. The difficulty is that simple laboratory conditions may have nothing to do with the reality of natural conditions. An example is provided by desiccation and starvation tolerance in *Drosophila*. Several laboratory investigations have repeatedly found a positive correlation between these two traits [19, 38, 39]. Studies of natural populations have shown, in contrast, a systematic negative correlation in several species, each apparently reacting adaptively to some environmental gradient related to latitude [7, 24].

If we argue that natural populations might be preferred to laboratory ones for evolutionary studies, a major problem to be raised is their stability. For example, several French populations of *D. melanogaster* were investigated with the isofemale line technique for size and other quantitative traits and slight but significant variations were shown between them [4]. Since the measurements were made for different years on lines sometimes kept in the laboratory for many generations, the origin of these variations has remained unknown. More recently, a significant difference in reaction norms of body pigmentation was demonstrated in two sibling species from two French localities, presumably reflecting, in that case, an adaptation to local thermal conditions [16].

In the present work we sampled twice, over a 5-year interval, the same population at the same time of the year and analysed two size-related traits, wing and thorax length. We also calculated the wing/thorax ratio, which is related to wing loading and flight capacity and might be a direct target of natural selection [34, 41]. Measurements were not restricted to a single laboratory condition, as was the case in former investigations. We analysed phenotypic plasticity related to growth temperature over the whole thermal range of the species. We found a remarkable stability not only of size but also of the reaction norms and of their genetic characteristics. Also a curvilinear, apparently quadratic variation of the genetic variance is shown according to growth temperature.

2. MATERIALS AND METHODS

Wild *D. melanogaster* adults were collected with banana traps in Grande Ferrade near Bordeaux (southern France) over 2 different years. A first collection was made in autumn 1992, and a second in 1997 from the same vineyard and same season. Isofemale lines were established and ten of them were randomly chosen for further study. For the 1992 sample, lines were kept for 5 months (6–7 generations) under laboratory conditions before being measured in April

1993. For the 1997 sample, measurements were made on the second laboratory generation in December 1997.

For investigating growth temperature effects, ten pairs of adults were randomly taken from each line and used as parents. They were allowed to oviposit at room temperature (20 ± 1 °C) for a few hours in culture vials containing a high nutrient medium based on killed yeast [8]. Such a medium prevents crowding effects which could affect fly size. Density ranged between 100 and 200 eggs per vial. These vials with eggs were immediately transferred to one of seven experimental temperatures (12, 14, 17, 21, 25, 28 and 31 °C). From each line at each temperature, ten females and ten males were randomly taken and measured for two quantitative traits (wing and thorax length) with a binocular microscope equipped with a micrometer. The results were expressed in mm × 100. Wing length was measured from the thoracic articulation to the distal tip of the wing, and the thorax was measured on a left side view from the neck basis to the tip of the scutellum [10, 28]. The wing/thorax ratio was also calculated.

A small experiment was performed from a mass culture to measure the effect of larval crowding on adult size. Larval density was controlled by transferring 10, 20, 40, 80, 160 and 320 eggs to culture vials. A still higher density (650 emerging adults) was obtained as a consequence of a large number of parents (50 females) directly laying in a single vial for a few hours.

Data were analysed with the Statistica software [43]. As in previous studies, the response curves were adjusted to polynomials [28]. For wing length, thorax length and wing/thorax ratio, a cubic polynomial was chosen for describing the norms. For genetic variance (V_g) and coefficients of genetic variation (CV_g), a quadratic polynomial was chosen. With cubic polynomials, numerous characteristic values can be calculated [11]. In the present case, we used the polynomial parameters to calculate the co-ordinates of a maximum, minimum or inflexion point, for wing and thorax length, V_g and CV_g or wing/thorax ratio, respectively.

3. RESULTS

3.1. Larval density and size variation

Figure 1 shows the relationship between larval density and wing or thorax length or wing/thorax ratio. A one-way ANOVA (not shown) on these data demonstrated significant differences for wing and thorax length but not for wing/thorax ratio. For wing and thorax length, however, the results became homogeneous (no effect of density) when the extreme values (densities of 10 and 650) were excluded from the analysis. We may conclude that a density range of 100 to 200 flies per vial will have no effect on the measured characters.

3.2. Mean reaction norms of wing and thorax length and wing/thorax ratio

The average response curves of size traits according to growth temperature are shown in *figure 2*. Female and male curves are separated showing the well-known fact that males are smaller than females. The major conclusion is that

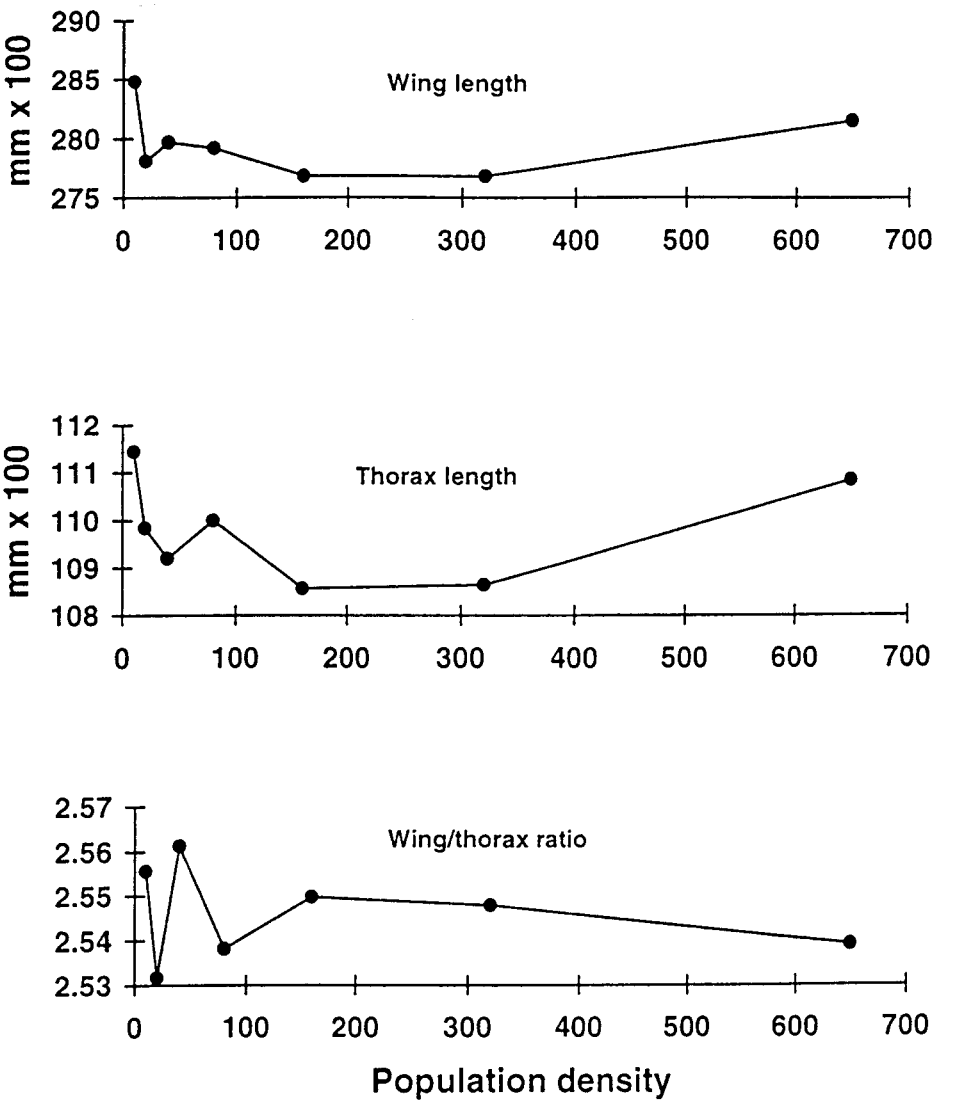

Figure 1. Variation of average wing length, thorax length and wing/thorax ratio according to population density in each culture vial. Each value is the mean of 25 observations.

for each trait, the reaction norms of years 1992 and 1997 are almost identical. For each character, a maximum was observed at low temperature, i.e. around 15 °C for wing length and 19 °C for thorax length, in agreement with previous studies [10, 28]. Reaction norms of wing/thorax ratio are given in *figure 3*. In both sexes a decreasing sigmoid was observed with only a slight difference between males and females. Data for the two samples were almost identical.

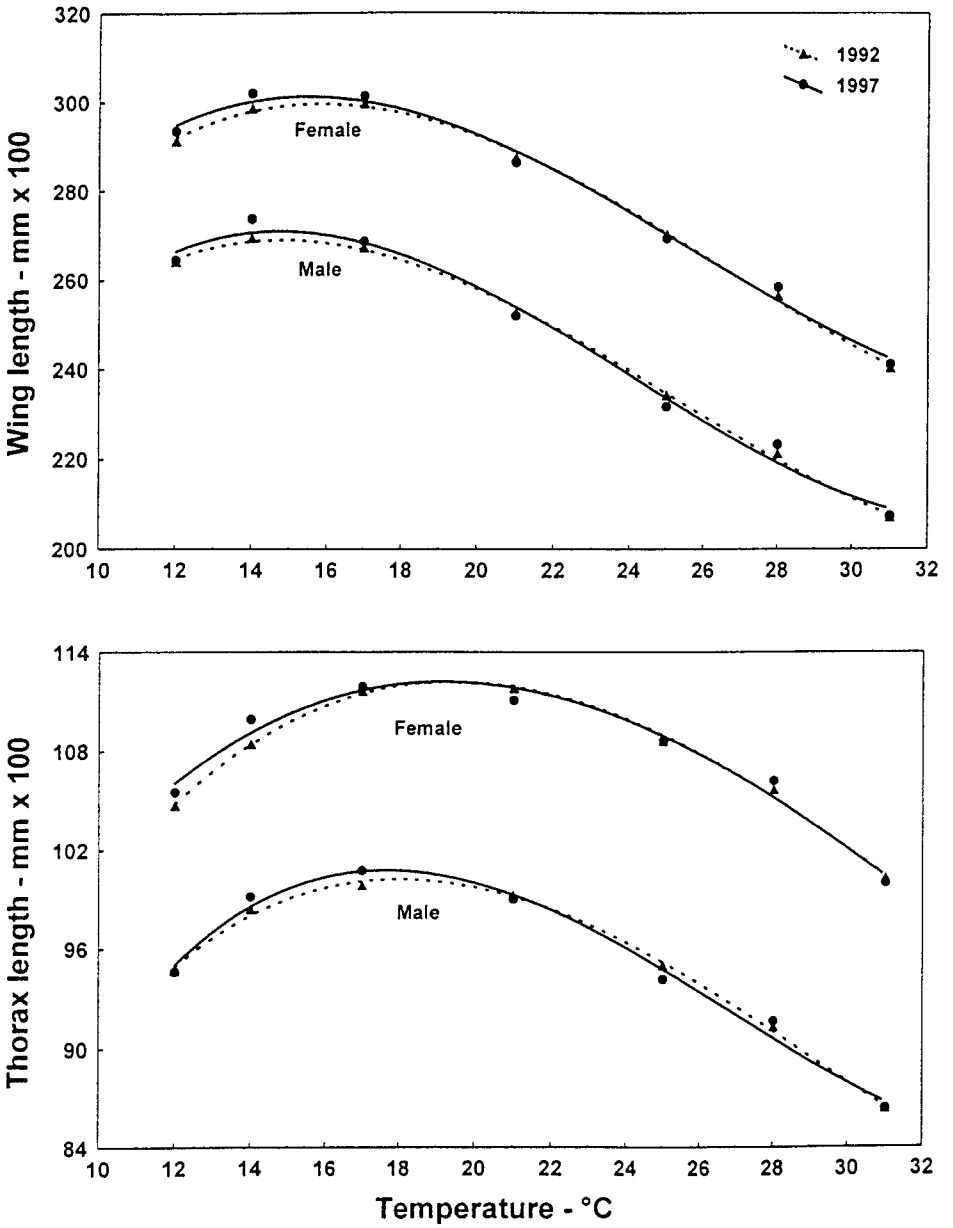

Figure 2. Average reaction norms of wing and thorax length in two samples from 1992 and 1997. In each case, experimental mean values are shown, as well as curves fitted to cubic polynomials.

The data were submitted to ANOVA, in which lines were considered as a random factor and nested within years: no significant differences were found between the years for each trait (*table I*). Significant differences were, however,

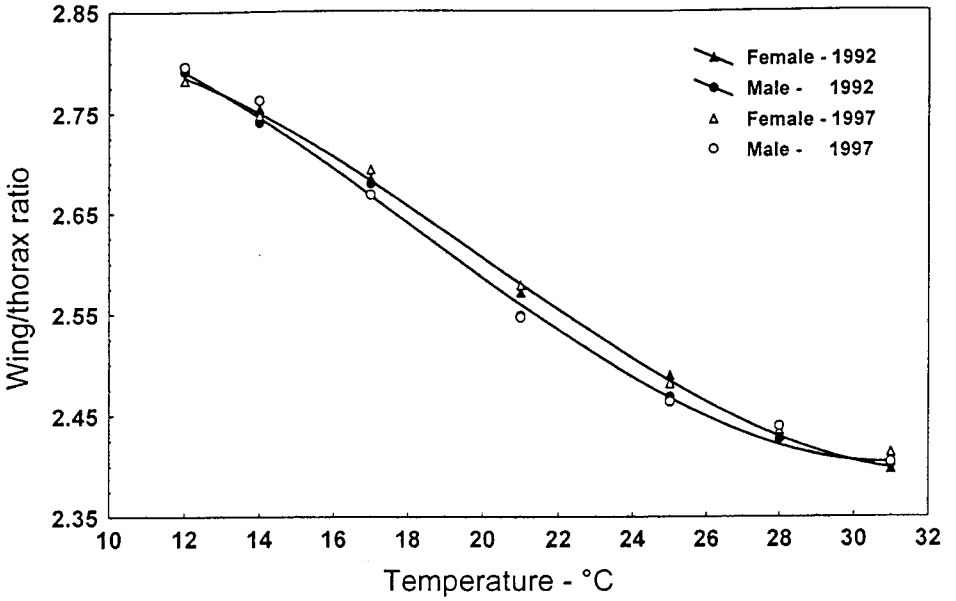

Figure 3. Average reaction norms of wing/thorax ratio in males and females in relation to growth temperature. The data of the 1992 and 1997 samples were so similar that only one average curve (1992) was drawn for each sex (cubic polynomial). Second sample (1997) values are represented by open symbols.

evidenced due to line, sex, temperature and their interactions. The interactions involving year were always non-significant. These analyses confirm the high similarity of the 2-year samples.

3.3. Characteristic values of reaction norms

As indicated previously, the response curves were adjusted to polynomials and the parameters were used to calculate characteristic values [11]. For the two concave norms (wing and thorax length), we considered only the co-ordinates of the maximum, i.e. MV (maximum value) and TMV (temperature of maximum value) (*table II*).

Maximum values were very similar between years. Coefficients of variation among lines were small and similar for both traits: 1.99 ± 0.13 for wing and 1.42 ± 0.19 for thorax length. Temperatures of maximum value were also similar for the two samples (*table II*). The data confirmed previous observations according to which TMVs were lower in males than in females and lower for wing length than for thorax length. Coefficients of variation were higher than for MV: 6.06 and 4.54 for wing and thorax, respectively. We finally compared the sigmoid norms of the W/T ratio by calculating the co-ordinates of the inflexion points (*table II*), that is, the phenotype at the inflexion point (PIP) and the temperature of the inflexion point (TIP). For this character, non-plausible values were found for some lines, for example, a PIP superior to 10 or a TIP of 50 °C. Such aberrant values were excluded from the calculations, so that only 34 values were available. Keeping only plausible values, we see

Table I. ANOVA applied on wing and thorax length, and wing/thorax ratio. Lines were considered as a random factor and nested in years.

Source of	df	Wing length		Thorax length		W/T ratio	
variation		MS effect	F	MS effect	F	Ms effect	F
Year (1)	1	556.3	0.17 (ns)	46.3	0.23 (ns)	0.0092	0.089 (ns)
Line (2)	18	3 226.5	98.48***	205.9	42.22***	0.1029	59.020***
Sex (3)	1	748 110.6	5 205.86***	108 228.0	5 057.01***	0.0387	6.868*
Temp. (4)	6	228 220.6	1 414.57***	8 062.7	362.76***	9.7199	1 381.927***
1 × 3	1	34.8	0.24 (ns)	4.8	0.23 (ns)	0.00001	0.001 (ns)
2 × 3	18	143.7	4.39***	21.4	4.39***	0.0056	3.232***
1 × 4	6	334.9	2.08 (ns)	26.8	1.21 (ns)	0.0037	0.532 (ns)
2 × 4	108	161.3	4.92***	22.2	4.56***	0.0070	4.035***
3 × 4	6	1 104.8	22.99***	294.6	42.59***	0.0183	6.074***
1 × 3 × 4	6	26.4	0.55 (ns)	10.7	1.55 (ns)	0.0066	2.175 (ns)
2 × 3 × 4	108	48.1	1.47**	6.9	1.42**	0.0030	1.731***
Error	2 520	32.8		4.9		0.0017	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = non-significant; df = degree of freedom.

Table II. Characteristic values of reaction norms in two samples of ten isofemale lines collected in 1992 and 1997. MV and TMV are maximum value and temperature of maximum value for wing and thorax length. Wing/thorax ratio is characterized by the phenotype at the inflexion point (PIP) and the temperature at this inflexion point (TIP). Mean values between years were never significantly different (Student *t*-test).

	Year: 1992		Year: 1997	
Trait/Sex	m ± S.E.	CV	m ± SE	CV
MV (mm × 100)				
Wing length				
Female	299.93 ± 2.07	2.19	301.84 ± 1.60	1.68
Male	269.31 ± 1.90	2.23	271.72 ± 1.63	1.90
Thorax length				
Female	112.24 ± 0.40	1.12	112.33 ± 0.69	1.94
Male	100.28 ± 0.36	1.13	101.03 ± 0.47	1.48
TMV (°C)				
Wing length				
Female	15.77 ± 0.21	4.16	15.26 ± 0.43	8.91
Male	14.72 ± 0.21	4.30	14.94 ± 0.32	6.76
Thorax length				
Female	19.47 ± 0.27	4.45	19.03 ± 0.34	5.63
Male	17.95 ± 0.18	3.25	17.80 ± 0.27	4.73
PIP: wing/thorax ratio				
Female	2.63 ± 0.02	2.41	2.63 ± 0.19	2.35
Male	2.68 ± 0.03	3.73	2.62 ± 0.01	0.85
TIP: wing/thorax ratio				
Female	18.71 ± 0.91	14.50	19.38 ± 0.79	12.94
Male	16.98 ± 1.20	21.25	19.16 ± 0.46	5.92

m: mean value; SE: standard error of the mean; CV: coefficient of variation.

that PIP were similar in males and females and also between samples (average 2.64). The temperatures of the inflexion point were not different between years (average 18.53 ± 0.48) but variability among lines was higher (average CV: 13.65).

3.4. Isofemale line heritabilities

Since we could not demonstrate any significant year effect, we pooled the data into a single sample of 20 lines in order to further analyse the genetic architecture of this Bordeaux population. Genetic variability was analysed by calculating, for each temperature and trait, the coefficient of intraclass correlation (*table III*) which estimates a broad sense heritability and is often considered as a specific parameter, i.e. isofemale line heritability [5, 15, 17, 18, 40]. ANOVA on these data (*table IV*) demonstrated a slight effect of sex (higher values in females) and of temperature (higher values at 14, 17 and 25 °C). A

Table III. Coefficients of intraclass correlation at various temperatures for wing and thorax length, and wing/thorax ratio in both sexes of *D. melanogaster*. Each value is calculated from a pool of 20 isofemale lines.

Temperature	Wing length		Thorax length		W/T ratio	
(°C)	Female	Male	Female	Male	Female	Male
12	0.48	0.30	0.30	0.25	0.36	0.26
14	0.56	0.53	0.43	0.29	0.37	0.49
17	0.48	0.55	0.46	0.33	0.33	0.47
21	0.44	0.46	0.38	0.20	0.48	0.37
25	0.55	0.46	0.41	0.39	0.49	0.32
28	0.57	0.36	0.38	0.26	0.36	0.33
31	0.42	0.40	0.28	0.26	0.20	0.35
Mean	0.50	0.44	0.38	0.29	0.37	0.37
SE	0.02	0.03	0.02	0.02	0.04	0.03

SE = standard error of the mean.

Table IV. ANOVA applied on intraclass correlation coefficients of wing length, thorax length and W/T ratio, according to growth temperature, sex and trait.

Source of variation	df	MS effect	F		% variation explained
Temperature (1)	6	0.017	3.55	*	25.16
Sex (2)	1	0.027	5.86	*	6.93
Trait (3)	2	0.069	14.73	***	34.84
1 × 2	6	0.007	1.43		10.16
1 × 3	12	0.002	0.33		4.68
2 × 3	2	0.008	1.71		4.04
1 × 2 × 3 (error)	12	0.005			14.19

* $P < 0.05$; *** $P < 0.001$.

major difference was found between traits, and especially a higher heritability of wing length, as already found by Capy et al. [5] with the same method.

3.5. Genetic variance and evolvability across temperatures

We calculated the genetic variance (see [17]) for each temperature, sex and trait. The results are illustrated in *figure 4*. In each case, higher values were observed at extreme high or low temperatures and lower values in the middle of the thermal range. As in Noach et al. [30], we adjusted these convex curves to a quadratic polynomial and calculated, in each case, a temperature of minimum value ($T_{\min V}$) (*table V*). Fairly high temperatures were found for wing length (average 27.9 °C) while $T_{\min V}$s were in the middle of the thermal range for thorax length and wing/thorax ratio (average 22.4 °C). For wing and thorax length, higher variances were observed in females, presumably in relation to their larger size (*figure 4*).

Table V. Temperature of minimum value ($T_{\min V}$) for genetic variance and genetic CV, for wing and thorax length, and wing/thorax ratio. Values were calculated with a quadratic polynomial adjustment. Goodness of fit is indicated by adjusted R^2.

Parameter	Wing		Thorax		W/T ratio	
	$T_{\min V}$	R^2	$T_{\min V}$	R^2	$T_{\min V}$	R^2
Genetic variance						
Female	26.33	0.92	21.63	0.86	22.78	0.80
Male	29.43	0.85	21.45	0.82	23.89	0.74
Genetic CV						
Female	22.35	0.83	21.14	0.91	21.89	0.79
Male	21.62	0.52	20.61	0.88	21.87	0.69

We also standardized the genetic variability to the mean value of each trait by calculating the genetic coefficients of variation (CV_g). The CV_g characterizes the capacity of a trait to respond to natural selection and was called evolvability [21, 22]. All these coefficients also exhibited convex response curves according to growth temperatures (*figure 5*). A significant difference between sexes persisted only for wing length. $T_{\min V}$s were all in the middle of the thermal range (*table V*) with an average of 21.6 °C.

We compared CV_gs with isofemale line heritability by analysing their correlations. In all six cases (traits and sexes) negative values were found ranging from – 0.15 to – 0.87 (average $r = -0.44 \pm 0.012$). These negative correlations are illustrated in *figure 6*. They show that heritability and evolvability do not provide the same biological information [21].

4. DISCUSSION AND CONCLUSIONS

We found a remarkable stability of the reaction norms of body size traits in two samples collected in the same place over a 5-year interval. This result was obtained using a high nutrient food, and a specific experiment showed that the results were not influenced by larval density. We may also suggest that experimental technique and food ingredients did not change over the years. Using such conditions, any significant difference between two samples could therefore be considered as reflecting a genetic divergence. In spite of the striking similarity of the average curves, each sample harboured a noticeable genetic variability between isofemale lines. The overall stability suggests, but does not demonstrate, that in a local population, size traits might be submitted to some stabilizing selection, not only for their mean value in a given environment but also for their reactivity to growth temperature. The fact that reaction norm shape may vary adaptively according to environmental conditions is demonstrated by major differences found between temperate and tropical populations [29].

Over the years polynomial adjustments of the reaction norms also established a remarkable stability of their characteristic values, either MV, TMV, PIP or TIP. Each value, which was calculated for each line by using the data of

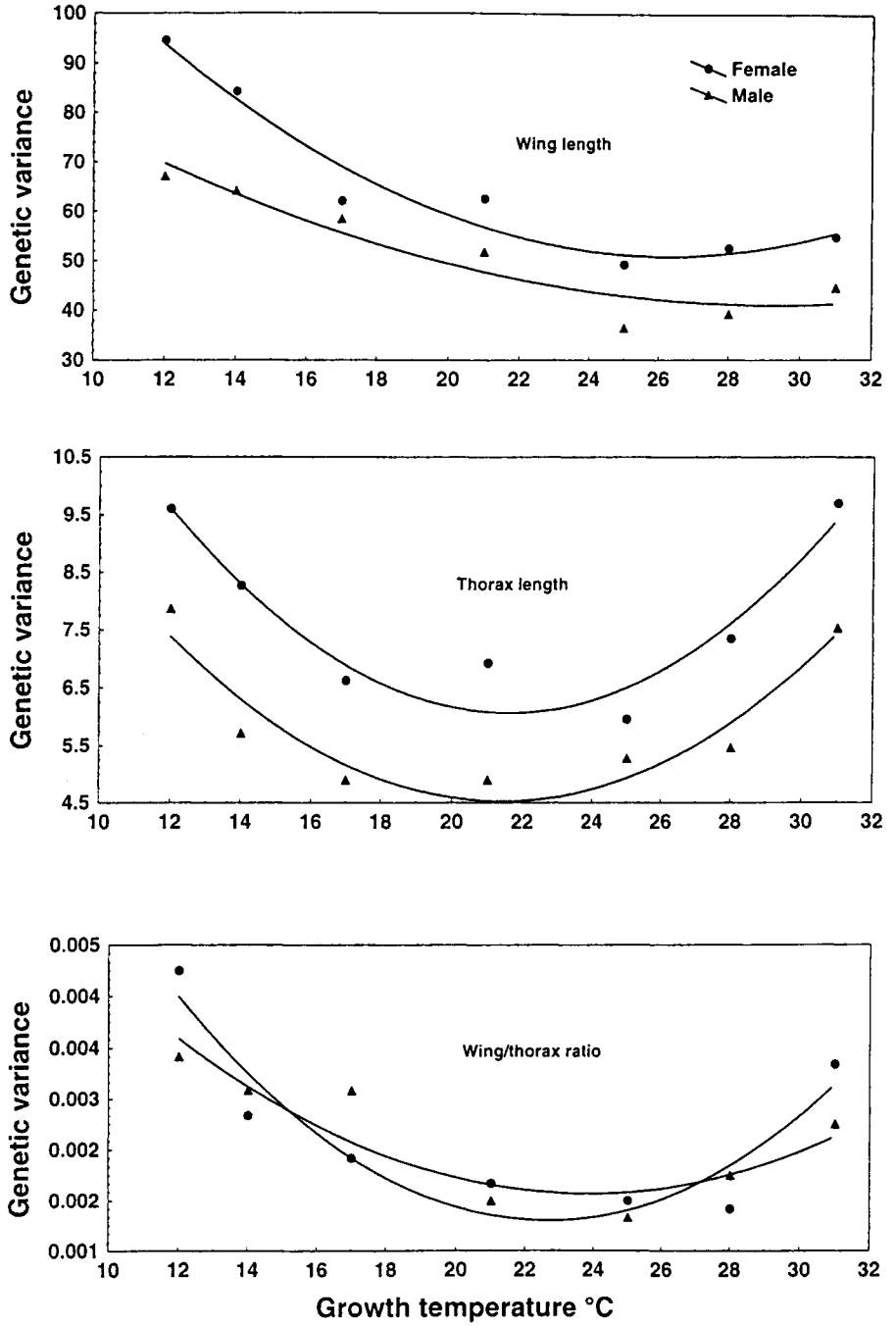

Figure 4. Variation of genetic variances according to growth temperature for wing length, thorax length and wing/thorax ratio. Note the higher variances in females for wing and thorax length, presumably due to a scaling effect (larger female size). Adjusted curves were drawn with a quadratic polynomial.

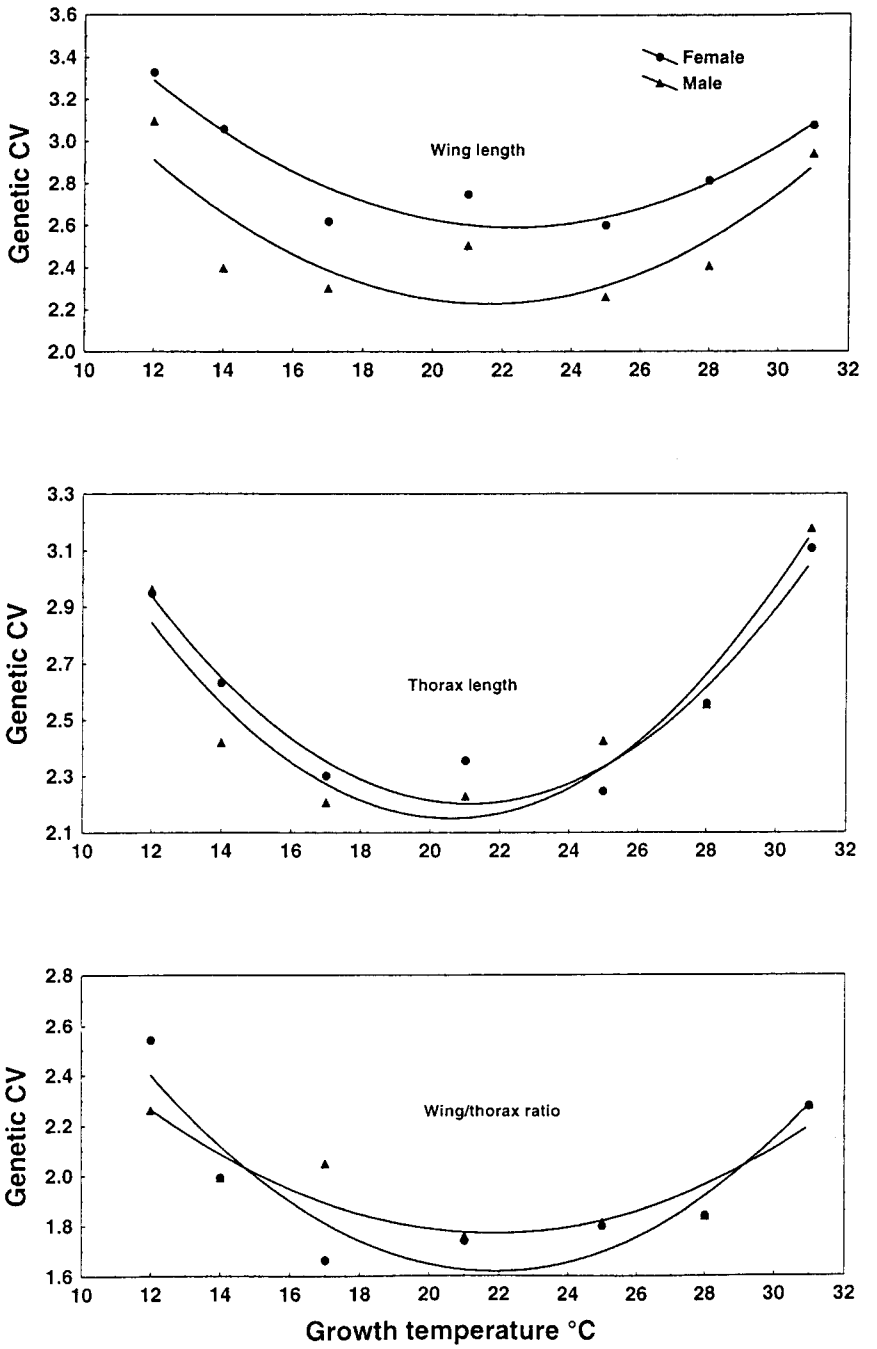

Figure 5. Genetic coefficients of variation (evolvability) according to growth temperature for wing length, thorax length and wing/thorax ratio.

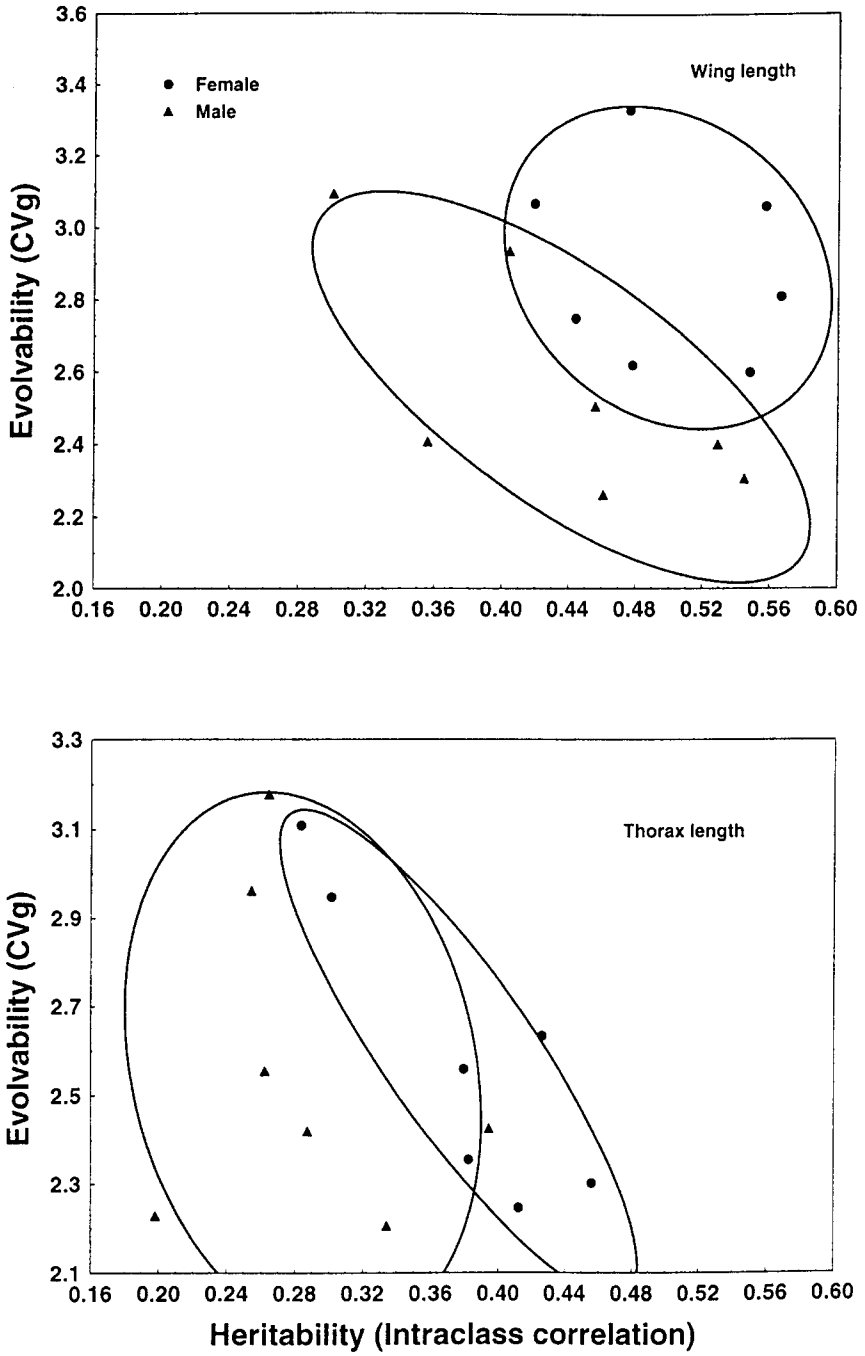

Figure 6. Negative correlations observed between heritability and evolvability of wing length and thorax length. Each point corresponds to one growth temperature.

70 individuals, is mainly a genetic property of the line. Sex differences also have a genetic basis. Using family means, we calculated the correlations between males and females at each temperature. No difference was found either between years or temperatures, with average values of 0.82 ± 0.05 for wing length and 0.70 ± 0.08 for thorax length.

Heritabilities (intraclass correlations) were significantly different among traits with higher values for wing length, in agreement with previous observations [5, 10]. Significant variations were also observed according to temperature but in a quite irregular way. Especially, there was no regular increase in heritability under extreme conditions as was suggested by other investigations [3, 20].

Genetic variances (V_g) varied according to growth temperature with higher values at low and high temperatures and a minimum around the middle of developmental range. Such a convex pattern was previously observed by Noach et al. [30] in a Tanzanian population and, interestingly, the temperature of minimum value was also less for thorax than for wing length. Noach et al. [30] failed, however, to find such a pattern in a French population. This contradiction may be explained by the fact that we used a broader thermal range (12–31 °C instead of 17.5–27.5 °C). According to de Jong [12, 13] and Scheiner [37], a minimum genetic variance should be expected at the predominant value of the environmental variable where the stabilizing selection pressure on the trait is the strongest. Our data on thorax length and wing/thorax ratio fit this expectation, since the minimum genetic variances are observed at temperatures around 22 °C which correspond to the summer temperature in the Bordeaux area. The identity of the average reaction norms over a 5-year interval also suggests that stabilizing selection might occur not only in the middle of the thermal range but also at other temperatures. This is likely to occur in Bordeaux, since low temperatures are experienced by spring and autumn generations.

It has been proposed that a higher genetic variance under extreme stressful conditions should permit a faster adaptive response to an environmental change [3, 12, 19]. However, as argued by Houle [21, 22], knowing the genetic variance and calculating heritability do not permit the speed of an adaptive change to be predicted. For this kind of prediction, it is better to standardize the genetic variance to the mean and estimate the evolvability of a trait by using the genetic coefficient of variation. We found that evolvability changed over temperature (*figure 5*) with minimum values at middle temperatures. In other words, evolvability was clearly higher under extreme environments so that adaptive changes should be faster under such conditions when needed.

Both heritability (intraclass correlation) and evolvability are ratios with the genetic variance as the numerator. It might be argued that both parameters estimate the same thing and should thus be positively correlated. We calculated these correlations separately for the three traits and two sexes. All the six coefficients were negative with a mean value of $r = -0.44 \pm 0.12$, significantly less than zero. Such a negative correlation is difficult to explain. It rules out, however, the above-mentioned possible bias of measuring the same thing twice. In the future, evolvability of a trait should receive increasing attention.

As discussed in the Introduction, laboratory evolution experiments, conducted by controlling some environmental factors, are certainly easier to in-

terpret in terms of selection although they might not be relevant to natural selection in nature. On the other hand, natural populations integrate so many environmental variables that their effects may be impossible to disentangle. As also discussed in detail by Rose et al. [36], laboratory experiments are plagued by a need for convenient controls. Flies collected in nature and brought to the laboratory are likely to undergo some rapid adaptation to general laboratory conditions such as a stable temperature, permanent food availability, early reproduction and absence of flight and dispersal. For that reason, numerous experiments were started from populations already kept as laboratory cultures [6, 31, 32, 35]. Laboratory evolution implies the establishment of aliquot strains under new conditions (e.g. different temperatures) while maintaining the initial ones. As stated by Rose et al. [36] "the best control may be perfectly preserved specimens from the founding population". Such a goal was attained on bacteria by keeping aliquot samples of the starting population frozen [26]. Our result, if it was generalized by further investigations, might provide a similar stable reference for *Drosophila*. In this respect, evolutionary experiments might encompass two kinds of controls: classical ones, kept under usual laboratory conditions, and wild living flies repeatedly sampled from the same locality.

ACKNOWLEDGEMENTS

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