

Evolutionary changes of nonlinear reaction norms according to thermal adaptation: a comparison of two *Drosophila* species

Changements au cours de l'évolution de normes de réaction non linéaires en fonction de l'adaptation thermique : comparaison de deux espèces de drosophiles

BRIGITTE MORETEAU*, JEAN-PHILIPPE MORIN, PATRICIA GIBERT, GEORGES PÉTAVY, ÉLIANE PLA, JEAN R. DAVID

Laboratoire populations, génétique et évolution, UPR 9034, CNRS, avenue de la Terrasse, bât. 13, 91198 Gif-sur-Yvette cedex, France

RÉSUMÉ

La signification adaptative des normes de réaction discontinues est généralement acceptée. En revanche, l'interprétation évolutionniste des normes continues reste difficile et l'existence de contraintes internes est souvent proposée pour expliquer les observations. Chez *Drosophila melanogaster*, divers caractères morphologiques présentent des normes convexes en fonction de la température, avec une valeur maximale comprise à l'intérieur de la gamme des températures compatibles avec un développement complet. Nous comparons une espèce adaptée au froid (*Drosophila subobscura*) pour laquelle le point thermique moyen est de 16 °C avec *D. melanogaster* adaptée au chaud (point moyen de 22 °C). Trois caractères ont été étudiés : la longueur de l'aile et du thorax dans les deux sexes et le nombre d'ovarioles des femelles. Les températures de valeur maximale des trois caractères se classent dans le même ordre dans les deux espèces : nombre d'ovarioles > longueur du thorax > longueur de l'aile. Des différences significatives ont aussi été observées pour les paramètres de courbure des ajustements polynomiaux quadratiques. L'observation principale est une translation latérale des normes de réaction : les valeurs maximales sont observées à des températures nettement plus basses chez l'espèce adaptée au froid. Ce résultat suggère un changement adaptatif des normes en fonction de la tolérance thermique des espèces. La sélection naturelle semble pouvoir agir non seulement sur les valeurs moyennes des caractères, mais aussi sur la plasticité phénotypique et la forme des normes de réaction.

Mots clés : *plasticité phénotypique, température de développement, longueur de l'aile, longueur du thorax, nombre d'ovarioles, Drosophila melanogaster, D. subobscura*

ABSTRACT

*While the adaptive significance of discontinuous reaction norms is generally accepted, the evolutionary interpretation of continuous response curves remains speculative, and the occurrence of internal constraints is often suggested as an explanation of experimental observations. In *Drosophila melanogaster*, various morphometrical traits exhibit convex reaction norms to growth temperature, with a maximum value within the developmental thermal range. We compared a cold-adapted species*

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*Correspondence and reprints

(*D. subobscura*) with a mid thermal range at 16 °C, to the warm-adapted *D. melanogaster* (mid thermal range at 22 °C) for three different morphometrical traits: wing and thorax length in both sexes and ovariole number in females. Maximum value temperatures were ordered in the same way for the three traits in both species: ovariole number > thorax length > wing length. Significant differences were also observed between the two species for the curvature parameter of the quadratic adjustment. The major observation was a significant lateral shift in the reaction norms: maximum values were observed at much lower temperatures in the cold-adapted species than in the warm-adapted one. The parallelism between mid thermal range variation and the position of the maximum value strongly suggests an adaptive displacement of the response curves. Natural selection may thus act not only on trait mean values but also on phenotypic plasticity and on the shape of reaction norms.

Key words: phenotypic plasticity, developmental temperature, wing length, thorax length, ovariole number, *Drosophila melanogaster*, *D. subobscura*

VERSION ABRÉGÉE

Drosophila subobscura est une espèce européenne adaptée au froid. Les variations de trois caractères morphologiques ont été analysées en fonction de la température de développement, sur toute la gamme possible, c'est-à-dire entre 6 et 26 °C. Les courbes de réponses, ou normes de réaction, ont été établies pour les mâles et les femelles et modélisées par des polynômes du second degré. Comme attendu, les paramètres polynomiaux se sont avérés très variables entre répétitions, et par ailleurs leur signification biologique est souvent difficile à comprendre. Ces paramètres permettent en revanche de calculer des points caractéristiques, et dans le cas présent les coordonnées du point maximum des normes convexes. Les résultats ont été comparés à ceux obtenus pour une population française de *D. melanogaster*, espèce d'origine tropicale et que l'on peut considérer comme adaptée à la chaleur (températures d'élevage possibles comprises entre 12 et 32 °C). Le résultat le plus intéressant est un déplacement latéral des nor-

mes de réaction : les températures de valeur maximale sont nettement plus basses chez l'espèce adaptée au froid. Ces températures ne sont pas les mêmes pour les trois caractères mesurés, mais l'ordre est le même chez les deux espèces. Le paramètre polynomial de courbure permet aussi de mettre en évidence des différences entre les deux espèces, mais le sens de celles-ci varie selon le caractère étudié. Enfin le rapport aile-thorax, qui est lié à la charge alaire et nous renseigne sur la capacité de vol, est peu différent entre les deux espèces. Ces résultats suggèrent que la valeur moyenne des caractères mesurés et la forme des courbes de réponse sont déterminées par des systèmes génétiques indépendants. La température met en jeu des gènes de régulation qui paraissent réagir, de façon adaptative, à l'environnement thermique. Ces gènes répondraient cependant moins rapidement à la sélection naturelle que les gènes agissant directement sur la valeur moyenne des caractères.

Introduction

Phenotypic plasticity, ie, the fact that a single genotype may produce different phenotypes in different environments, is receiving increasing attention from evolutionists [1–9]. In this respect, two types of reaction norms can be considered. A first case concerns discontinuous variations leading to two different phenotypes with a regulatory threshold. The adaptive significance of the two phenotypes is generally assumed. Examples of such discontinuous reaction norms include enzymatic adaptation in bacteria, diapausing and non-diapausing stages in insects, winged and wingless forms in aphids [2, 6, 10]. A second, more general case, corresponds to a quantitative and progressive variation of the phenotype along an environmental gradient. Since most biological systems are expected to react to environmental changes, the significance of continuous reaction norms is less clear [3, 4, 11]. The response curve may be considered as a consequence of complex interactions between internal and external constraints, without any direct adaptive significance [3].

Several investigators have tried to model the evolution of reaction norms, generally considering two environ-

ments for the sake of simplicity [7, 12–17]. Also many experimental analyses have been restricted to two or three environments only [1, 18–20].

Two major questions concerning continuous reaction norms need to be addressed by experimental investigations. i) Is the shape of a reaction norm an adaptive response to the environment or a contingent process? ii) Are there specific genes regulating the shape of the norm and independent of the trait mean value? The problem is especially complicated when nonlinear norms are considered. A recent controversy shows that we are still far from a general consensus [11, 21–23].

For *Drosophila* as for all ectothermic organisms, temperature is a most important component of the environment. Its evolutionary role is demonstrated by different geographical distribution among species [24], by the occurrence of latitudinal clines for various traits in several species [25], and by quantitative divergence between laboratory populations kept under different thermal conditions [25–27]. Numerous morphometrical traits are modified according to growth temperature, and when a broad range of temperatures has been used, nonlinear norms have been obtained [25, 28–31]. Practically, two

main types of curves are observed: curves with a single maximum or minimum, and curves with a sigmoidal shape.

A recent investigation on ovariole number [30] in temperate and equatorial populations of *D. melanogaster* confirmed major differences in mean number, in agreement with a well-known latitudinal cline [32]. The shapes of the curves, with a maximum at intermediate temperatures, were however very similar. A very small difference was observed in the position of the maximum value, occurring at 22.2 °C in the temperate population, and at 22.7 °C in the equatorial one. These observations suggested that natural selection has been more efficient in changing the mean value of this fitness-related trait than its reactivity to temperature.

The time of divergence between the two geographical races might be too short to allow an efficient adaptive transformation of the shape of the reaction norms. In this respect a comparison of different species, isolated for a longer evolutionary time and adapted to different thermal environments, should provide a better insight on the possible role of natural selection in shaping reaction norms. In the present work, we investigated the European *D. subobscura* over its whole developmental thermal range. Data on this cold-adapted species were compared to those of *D. melanogaster*, which is native to the Afrotropical region and can be considered as warm-adapted [33]. We have found that, for three different morphological traits, maximum values were observed at much lower temperatures in *D. subobscura* than in *D. melanogaster* [29], suggesting an adaptive modification of the reaction norms.

Material and methods

D. subobscura population

A wild living population was collected in Montgenèvre (French Alps) at an altitude of 1 800 m in July 1993. For this investigation, we used the isogroup technique (see [34]). Six groups of ten wild collected pairs were set in culture vials to initiate parallel and independent replicates of the same population. These groups were then kept in bottles as laboratory mass cultures at 18–20 °C.

Temperature experiments

Adult flies from each group were used as parents for the temperature experiments. Parent flies oviposited directly in food vials, which were transferred to the experimental temperatures (5–27 °C). Since *D. subobscura* is quite difficult to breed in the laboratory, some experiments had to be repeated on successive generations especially at low temperatures. We used a high nutrient, killed yeast food for all experiments.

Traits measured and data analysis

Wing and thorax lengths were measured with a binocular microscope and micrometer values converted into mm \times 100. The wing/thorax ratio was also calculated for each fly. Ovariole number (both ovaries) was determined by dissection of 1-week old females. For each group, ten males and ten females were randomly taken and measured for each experimental temperature. Little genetic variation is expected between groups, and each of them may be considered as an experimental repetition [34]. For each trait, nonlinear norms were adjusted to a quadratic polynomial [17, 30]:

$$y = g_0 + g_1t + g_2t^2$$

where y is the phenotype at the t developmental temperature and g_0 , g_1 and g_2 are polynomial parameters.

The polynomial parameters were found, as in a previous paper [30], to be highly variable and difficult to interpret. Such a polynomial is however equivalent to $y = MV + g_2(t - TMV)^2$, where MV and TMV refer to the maximum value of the trait and to the temperature of that maximum. Such characteristics have clearer biological meaning and are useful for statistical comparisons. In the case of the wing/thorax ratio, a third power polynomial had to be used as in *D. melanogaster*. Mathematical adjustments were made with the Statistica software [35].

D. subobscura data were compared to those obtained, with similar rearing and measurement techniques, on ten isofemale lines from a French natural population of *D. melanogaster* [29].

Results

The developmental thermal range of *D. subobscura*

This species is known to be sensitive to high temperature [36] but no detailed information was available. In the course of this study, we found that the upper limit for a complete development was close to 26 °C. At 27 °C, a high mortality, over 90%, was always observed. Data for wing and thorax length could still be obtained for that temperature. Ovariole number, however, could not be measured in all groups owing to premature death of most emerging females. On the cold side of the thermal range, it progressively appeared that a normal development with a low mortality was possible below 10 °C. Laboratory cultures of all groups were obtained at 9, 8, 7 and 6 °C. The temperature of 5 °C was close to the lower limit. Complete development, lasting about 6 months, was observed in some groups but it was not possible to obtain the 60 adults of each sex as for other temperatures. We thus consider that the possible thermal range of *D. subobscura* at constant temperatures lies between 6 and 26 °C, with a mid point at 16 °C. This contrasts with *D. melanogaster* for which the limits are 12–32 °C [37], and the mid value 22 °C.

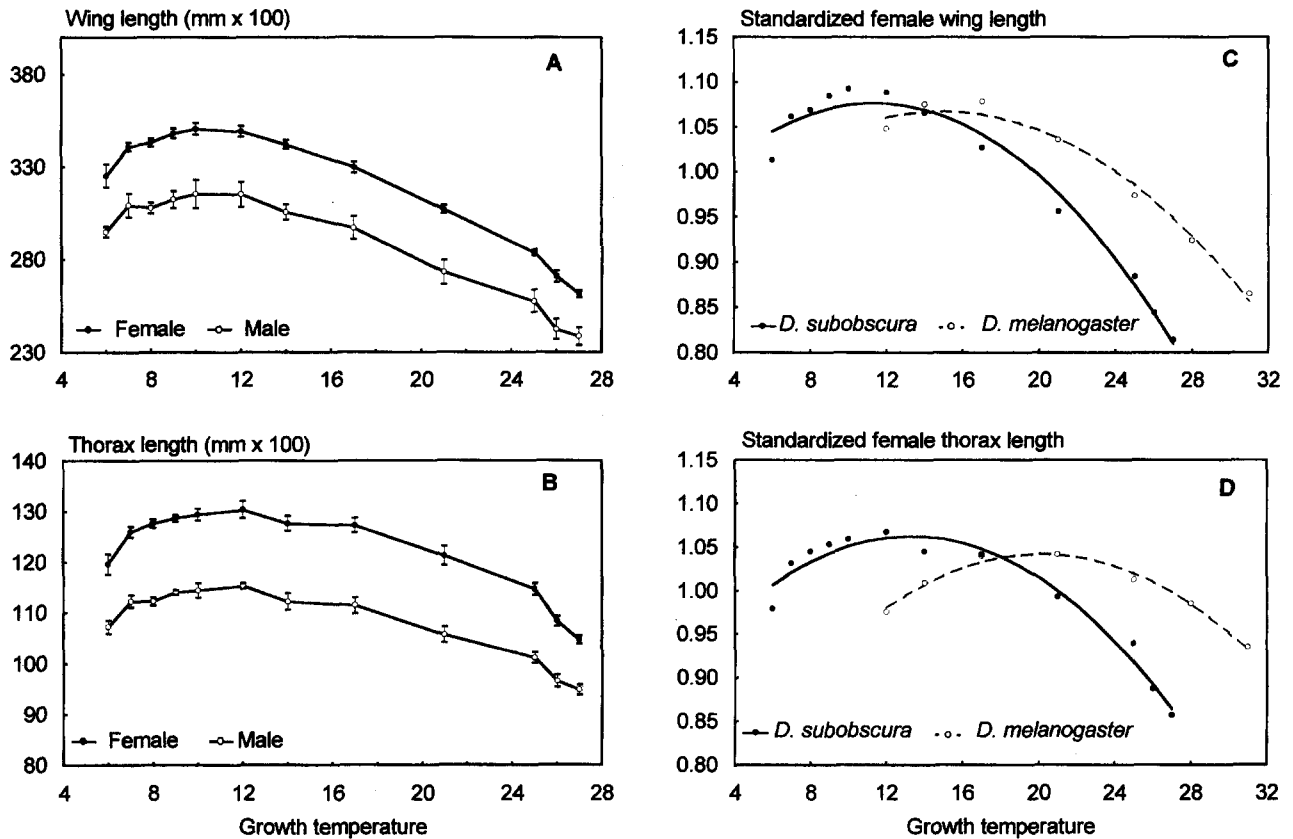


Figure 1. Average reaction norms of wing (A) and thorax (B) length in *D. subobscura* males and females.

Vertical bars indicate the 95% confidence intervals.

(C) and (D): Comparison of the shape of the norms in *D. subobscura* and *D. melanogaster*.

Data were standardized to the same overall mean (= 1). Experimental points are shown as well as the adjusted curves from a quadratic polynomial.

Reaction norms of wing length, thorax length and ovariole number

Average curves of wing and thorax length (figure 1A and B) show that, in *D. subobscura* as in *D. melanogaster* [29], males are smaller than females. For both traits, mean values are higher at low temperature, around 10 °C for wing and 12 °C for thorax. *D. subobscura* is bigger than *D. melanogaster*. For a visual comparison of the two species, data were standardized, ie, adjusted to the same overall mean of 1. Adjusted curves, given for females only (figure 1C and D), reveal a major difference due to a lateral translation of the reaction norms. This point will be statistically analyzed in a further section. The average curve of ovariole number (figure 2A) also exhibits a convex shape with a maximum at a low temperature around 13 °C. The comparison of standardized data with *D. melanogaster* (figure 2B) again evidences a lateral difference of the response curve of *D. subobscura* towards lower temperatures.

The wing/thorax ratio

The reaction norm of this trait (figure 3A) corresponds to a decreasing sigmoid curve. In *D. subobscura*, a signifi-

cant sex dimorphism was observed with significantly higher values in males, on average 2% above those of females. In *D. melanogaster*, the wing/thorax ratio was almost identical in the two sexes: on average 0.3% less in males [29]. A comparison of the two species (figure 3B) shows that the curves exhibit similar sigmoidal shape, although the average slope is steeper in *D. melanogaster*.

Statistical comparisons between *D. subobscura* and *D. melanogaster*

Polynomial adjustments were made for the various traits, allowing the calculation of critical values, which are easier to interpret. For the traits adjusted to a quadratic equation, ie, wing and thorax length and ovariole number, we considered three characteristics: the temperature of the maximum value (TMV), the maximum value at that temperature (MV), and the g_2 parameter, which measures the curvature of the norm. For the wing/thorax ratio we considered two characteristics only, ie, the temperature of the inflexion point (TIP) and the value of the ratio at that temperature (mean ratio).

Numerical values are given in table I and compared to those calculated for *D. melanogaster*. MVs for wing and

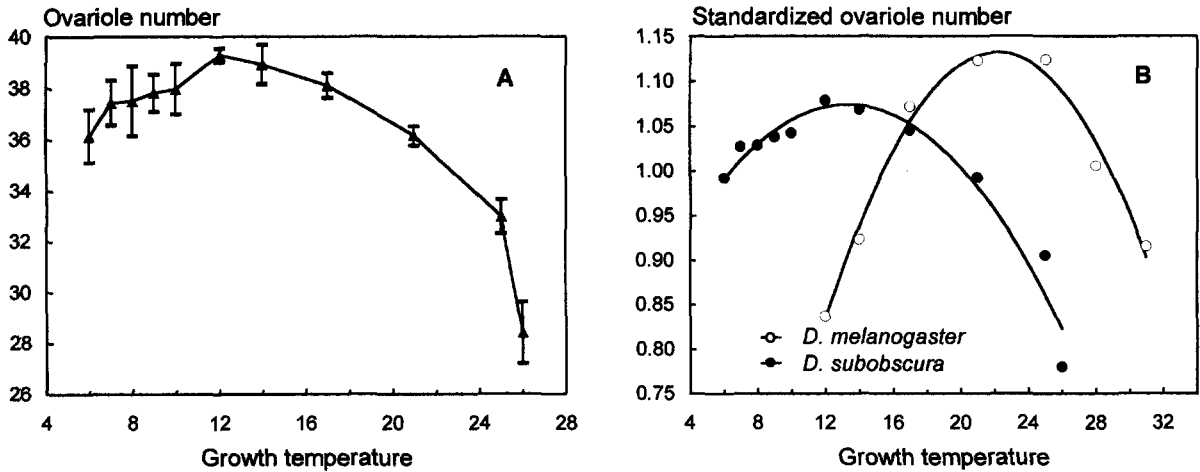


Figure 2A. Average norm of reaction of ovariole number in *D. subobscura*.

Vertical bars indicate the 95% confidence intervals.

B. Comparison of the standardized norms in *D. subobscura* and *D. melanogaster*.

Experimental points and quadratic polynomial adjustments are shown.

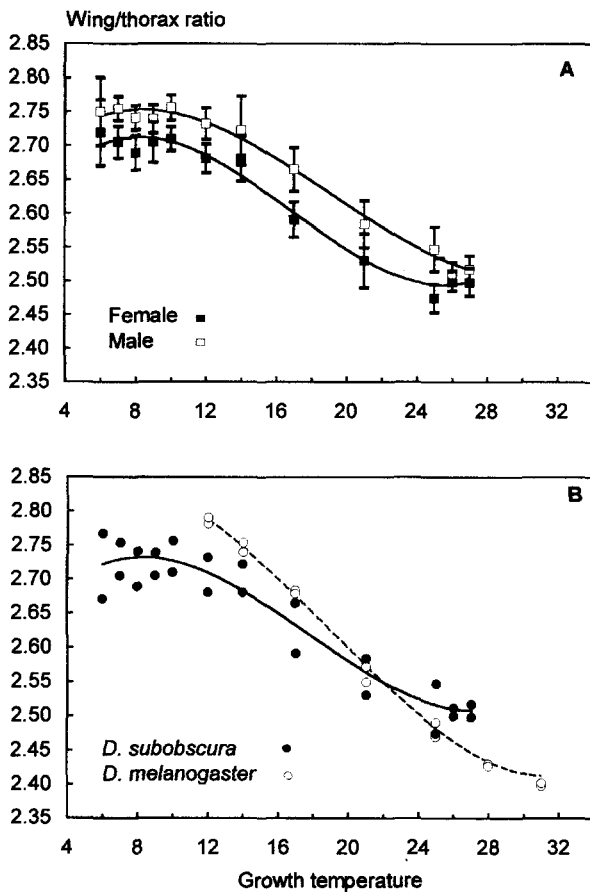


Figure 3A. Reaction norms of wing/thorax ratio in *D. subobscura*.

Experimental values and curves adjusted with a cubic polynomial are shown. Male ratios are significantly higher than female ratios for several temperatures.

B. Comparison of the norms in *D. subobscura* and *D. melanogaster*.

For each temperature, values of males and females are shown; adjusted curves are the average of both sexes.

thorax clearly show that *D. subobscura* is a bigger species than *D. melanogaster*. The ovarian size is however less in the former species. These maximum values are characterized by small coefficients of variation (CV), (on average 1.73 ± 0.46).

TMVs (table I) are significantly different among traits, with the following order: wing < thorax < ovarioles, which is the same in the two species. For wing and thorax length, male maximum size is observed at a lower temperature than in females. A more interesting observation is that, in all cases, TMVs are lower in *D. subobscura*, ie, in the cold-adapted species. The differences are highly significant except for male wing length. For all TMVs, variability among groups (*D. subobscura*) or lines (*D. melanogaster*) is generally higher than for MVs, with average CVs of 5.46 ± 0.51 and 8.45 ± 3.74 for *D. subobscura* and *D. melanogaster*, respectively. The divergence among TMVs of the two species is illustrated in figure 4. Some overlap exists for TMVs of wing length, while a complete species separation is observed for TMVs of thorax length and ovariole number. The difference in reaction norms between the two species is well illustrated by the scatter-plot of TMVs for thorax length and ovariole number (figure 4).

The curvature parameters g_2 are much more variable among groups or lines (table I) than the coordinates of characteristic points, as already found in a previous paper dealing with ovariole number [30]. Average CVs were 20.51 ± 4.12 and 13.81 ± 1.88 in *D. melanogaster* and *D. subobscura*, respectively. For thorax length, lesser absolute values of g_2 are found in males of both species, with little difference between them. For wing length, males again exhibit lesser absolute values than females, with also a difference between species corresponding to

Table I. Quantitative characteristics of reaction norms in *D. subobscura* and *D. melanogaster* and comparison between the two species.

		<i>D. subobscura</i>		<i>D. melanogaster</i>		Comparison (<i>t</i>)	
		<i>m</i> ± <i>SE</i>	<i>CV</i>	<i>m</i> ± <i>SE</i>	<i>CV</i>	<i>d</i> ± <i>SE</i>	<i>P</i>
<i>Maximum values</i>							
Wing (mm × 100)	♀	345.66 ± 0.94	0.67	297.15 ± 2.33	2.48	48.51 ± 3.14	***
	♂	310.29 ± 0.75	0.59	268.81 ± 2.28	2.68	41.48 ± 3.04	***
Thorax (mm × 100)	♀	129.86 ± 0.47	0.90	111.89 ± 0.37	1.04	17.97 ± 0.60	***
	♂	113.96 ± 0.42	0.90	99.58 ± 0.33	1.05	14.38 ± 0.54	***
Ovarioles	♀	39.15 ± 0.27	1.69	48.43 ± 0.81	5.29	9.28 ± 1.08	***
<i>Temperatures of maximum value</i>							
Wing	♀	11.33 ± 0.26	5.60	14.70 ± 0.50	10.69	3.37 ± 0.68	***
	♂	10.76 ± 0.19	4.27	11.26 ± 0.79	22.08	0.50 ± 1.04	NS
Thorax	♀	13.21 ± 0.30	5.58	20.12 ± 0.17	2.60	6.91 ± 0.31	***
	♂	12.35 ± 0.36	7.22	18.47 ± 0.28	4.85	6.12 ± 0.46	***
Ovarioles	♀	13.26 ± 0.25	4.62	22.17 ± 0.14	2.02	8.91 ± 0.26	***
<i>Curvature parameters g_2</i>							
Wing	♀	-0.351 ± 0.016	11.19	-0.227 ± 0.013	18.01	0.124 ± 0.021	***
	♂	-0.283 ± 0.010	8.51	-0.169 ± 0.010	18.37	0.114 ± 0.015	***
Thorax	♀	-0.129 ± 0.007	13.58	-0.101 ± 0.004	13.62	0.028 ± 0.018	**
	♂	-0.092 ± 0.007	18.93	-0.080 ± 0.009	36.65	0.012 ± 0.013	NS
Ovarioles	♀	-0.057 ± 0.004	16.86	-0.125 ± 0.006	15.92	0.068 ± 0.009	***
<i>Wing/thorax ratio</i>							
Temperatures of inflexion point	♀	16.67 ± 0.25	3.65	19.05 ± 0.84	14.02	2.38 ± 1.23	NS
	♂	18.42 ± 0.66	8.72	16.93 ± 1.18	21.97	1.49 ± 1.77	NS
Mean values at TIP	♀	2.600 ± 0.002	0.18	2.623 ± 0.025	2.80	0.023 ± 0.033	NS
	♂	2.634 ± 0.007	0.59	2.681 ± 0.035	3.90	0.047 ± 0.048	NS

Results are the average values of six groups in *D. subobscura* and ten lines in *D. melanogaster*. *m*: Mean value; *SE*: standard error; *CV*: coefficient of variation; *d*: difference between species; comparison made with Student's *t* test: NS: non significant; ****P* < 0.01; ***P* < 0.001.

a stronger curvature of the reaction norm in *D. subobscura*. For ovariole number, a highly significant difference also exists between the two species but in the opposite way, ie, a lesser curvature in *D. subobscura*, as visualized in figure 2B.

The wing/thorax ratio, which was adjusted to a cubic polynomial, has only been characterized in the present work by the temperature of inflexion point (TIP) and the mean value at that temperature. No statistically significant differences between the species were found, either for the TIPs or the mean values at the TIP. In *D. melanogaster*, the mean TIP for both sexes lies clearly below the middle of the thermal range (18 versus 22 °C) while in *D. subobscura* it is above this point (17.5 versus 16 °C).

Discussion and conclusions

Since *D. melanogaster* is native to tropical Africa while *D. subobscura* is restricted to the temperate palearctic region, it was inferred that the former should be more tolerant to warm temperature than the latter. Laboratory experiments confirmed this expectation. While the thermal ranges of the two species have the same breadth (20 °C), the midpoints are highly contrasted with a difference of 6 °C (16 versus 22 °C) and *D. subobscura*

appears as cold-adapted. Both species are known to react to climatic conditions by producing latitudinal or altitudinal adaptive clines for various traits [25]. In the present study, a montane population of *D. subobscura* from the French Alps was chosen, and this fact might have contributed to its cold tolerance. A few experiments were made with another French population from the Paris vicinity and its upper lethal point was found to be at around 27 °C, similar to the value found for the Alpine population. For *D. melanogaster*, a temperate population was also used, and, in that case, the thermal ranges of tropical and temperate populations are known to be almost identical [37].

Nonlinear reaction norms to growth temperature have been observed for the three morphometrical traits. In both species, wing length, thorax length and ovariole number produce convex norms, which are conveniently described by quadratic polynomials. In evolutionary biology, maximum values of fitness-related traits are often considered as optimums [3]. In *Drosophila*, size characters and ovariole number are fitness-related traits (see discussion in [29, 30]), a higher adult fitness being provided by a larger body size or more numerous ovarioles. Since maximum values are observed at different temperatures for different traits, it is not possible to argue for a single adaptive optimization. Some internal constraints, involving development and phylogeny, might explain the different TMVs of the various traits.

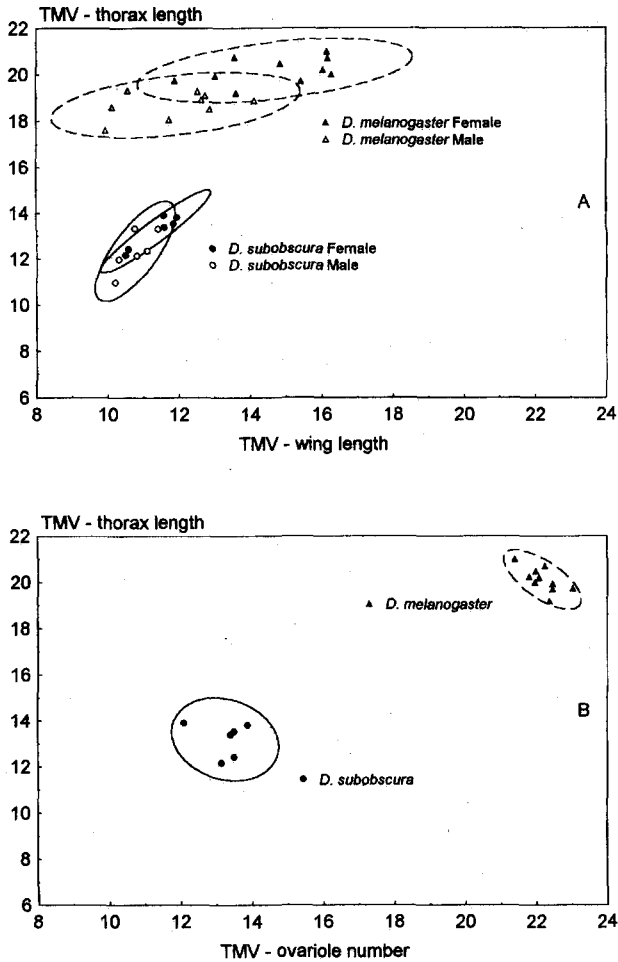


Figure 4A and B. Scatterplots of TMVs (temperatures of maximum value) in *D. subobscura* and *D. melanogaster*.

Each point corresponds to an isofemale line in the case of *D. melanogaster* and to an isogroup in *D. subobscura*. Ellipses comprising 95% of the observations are also shown.

The fact that size characters exhibited a nonlinear norm with respect to growth temperature has been known for a long time [38]. The difficulty in interpreting such curves in evolutionary terms generally led investigators to consider that they were deprived of any adaptive significance. Indeed several authors who investigated phenotypic plasticity of size generally considered a restricted thermal range, above the TMV, so that a more or less regular monotonous decrease of size could be observed [19, 20, 27]. In such a restricted range, a parallelism exists between the size reduction due to increasing growth temperature and the decrease of size observed in latitudinal clines, which is also correlated to increasing ambient temperature [32].

Our data demonstrate that internal developmental constraints in *Drosophila* species were not strong enough to prevent a modification of the shape of the reaction norms, and especially of the position of TMVs. A shift in the posi-

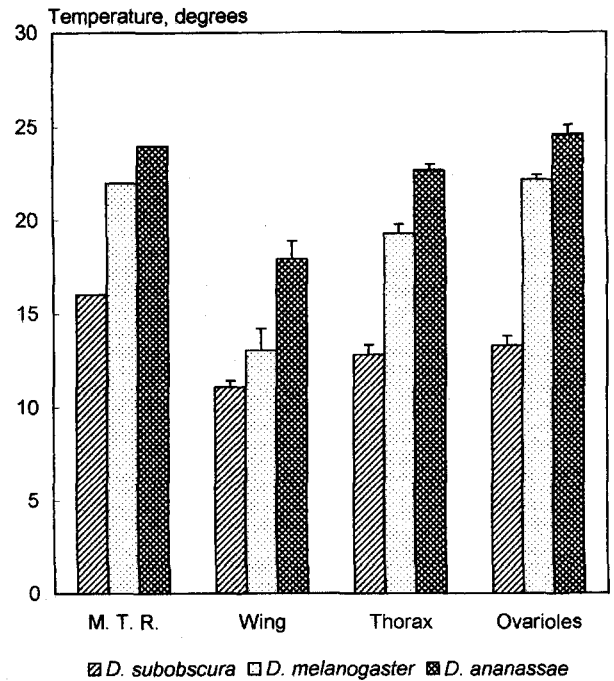


Figure 5. Parallel variations between the middle of the thermal range (M.T.R.) and the temperatures of maximum value (TMVs) for wing length, thorax length and ovariole number (both sexes averaged for wing and thorax).

Vertical bars indicate the 95% confidence intervals. Data on the cold-sensitive *D. ananassae* [39] are included for comparison with *D. subobscura* and *D. melanogaster*.

tion of the maxima toward lower temperatures has been observed in the cold-adapted species. The parallelism observed between the variation of the thermal range and the translation of the TMVs is illustrated in figure 5. The adaptive argument is reinforced by considering data on the tropical *D. ananassae* [39], which is cold-sensitive (lower possible growth temperature of 16 °C). In that species, a shift of the TMVs is observed but in the opposite direction, ie, at warmer temperatures than in *D. melanogaster* (figure 5).

The wing/thorax ratio, which is negatively correlated to wing loading and could be involved in flight capacity [29, 40, 41], exhibits a decreasing sigmoid reaction norm in both species. A significant difference between the two species concerns the sexual dimorphism found in *D. subobscura*, while identical values are observed for the two sexes in *D. melanogaster*. For the moment, no clear interpretation of this variation can be proposed. On the other hand, the reaction norms of the wing/thorax ratio are quite similar in the two species, both for the mean values and for the general shape. This similarity may be interpreted in two opposite ways: either it means that the wing/thorax ratio is not directly modified by the thermal adaptation, or that it is a highly adaptive trait responding rapidly to natural selection. According to the latter interpretation, the similarities between the two species

could be due to the fact that we investigated a temperate *D. melanogaster* population. In favor of this interpretation we may indicate that tropical populations of *D. melanogaster* are characterized by significantly lower values of their wing/thorax ratio (unpublished results).

A last but major issue for the evolution of reaction norm is the nature of the genes involved in the regulation of the shape. In a recent series of investigations Scheiner [4], considering only linear norms, argued about the existence of two kinds of genes, some regulating the trait mean and others determining plasticity, ie, the slope of the norm. This hypothesis resulted in a recent controversy [11, 21], Via arguing against the occurrence of two types of genes.

The comparison of the two species shows that, after a long evolutionary separation, mean trait values have diverged, *D. subobscura* being larger than *D. melanogaster* but with a lower ovariole number. Such varia-

tions implemented genes acting on trait mean value and confirm a genetic independence between body size and ovariole number, already known in *D. melanogaster* [42]. Variations in the position of the TMVs are easier to explain by assuming that specific regulatory genes were responsible for the lateral shift, in a probably adaptive way. Whether the same set of genes is able to regulate the different traits or whether each trait needs specific genes remains to be investigated. Also the curvature parameter g_2 , which somehow measures a general reactivity to temperature, might be related to other regulatory genes. Our data illustrate the complex determinism of nonlinear norms, the possibility of analyzing them by polynomial adjustments and the probable adaptive significance of some shape modifications. A comparison of more numerous species with different body sizes and various thermal characteristics should now be undertaken.

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