RESEARCH ARTICLE

Adaptation to different climates results in divergent phenotypic plasticity of wing size and shape in an invasive drosophilid

ROBERTA LOH[1], JEAN R. DAVID[2], VINCENT DEBAT[3] and BLANCHE CHRISTINE BITNER-MATHÉ[1]*

[1]*Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro 21944-970, Brazil*
[2]*Laboratoire Evolution, Génome, Spéciation, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette, France et Université Paris Sud 11, 91405 Orsay cédex, UMR5202 Paris, France*
[3]*Museum National d'Histoire Naturelle, Départment Systématique et Evolution, UMR5202 Paris, France*

Abstract

The phenotypic plasticity of wing size and wing shape of *Zaprionus indianus* was investigated in relation to growth temperature (17°C to 31°C) in two natural populations living under different climates, equatorial and subtropical. The two populations were clearly distinguished not only by their wing size (the populations from the colder climate being bigger in size), but also by the shape of the response curves to growth temperature i.e., their reaction norms. In this respect, the temperature at which the size of the wing was maximum was about 3°C higher in the equatorial population. Such a difference in size plasticity is already found in two other nonclosely related species, might be a general evolutionary pattern in drosophilids. Wing shape was investigated by calculating an ellipse included into the wing blade, then by considering the ratio of the two axes, and also by analysing the angular position of 10 wing-vein landmarks. For an overall shape index (ratio of the two axes of the ellipse), a regular and almost linear increase was observed with increasing temperature i.e., a more round shape at high temperatures. Wing shape was also analysed by considering the variations of the various angles according to temperature. A diversity of response curves was observed, revealing either a monotonous increase or decrease with increasing temperature, and sometimes a bell shape curve. An interesting conclusion is that, in most cases, a significant difference was observed between the two populations, and the difference was more pronounced at low temperatures. These angular variations are difficult to interpret in an evolutionary context. More comparative studies should be undertaken before reaching some general conclusions.

Introduction

Temperature is a major factor of the environment for explaining the distribution and abundance of animals (Andrewartha and Birch 1954; Precht *et al.* 1973; David *et al.* 1983; Cossins and Bowler 1987; Leather *et al.* 1993; Hoffmann and Parsons 1997). When a species lives under a very different climatic conditions, it adapts to the local climate, and this often results in progressive genetic variations among populations, such as latitudinal clines. On the other hand, phenotypic plasticity is a general property of living beings (Schlichting and Pigliucci 1993; Pigliucci 1996, 2001; DeWitt and Scheiner 2004).

As a first approximation, the fact that the same genotype can produce different phenotypes might appear an obstacle to evolution by natural selection. It might, however, contribute to geographical adaptation itself, if there is genetic variation for plasticity.

The way a phenotype behaves along an environmental gradient is determined by its genotype. In other words, genetic studies should be conducted to compare the response curves to an environmental gradient of different populations, i.e. the shape of the reaction norms.

In the drosophilid family, which now comprises more than 3500 described species, most of them can be described as either tropical (cold sensitive) or temperate (cold tolerant but heat sensitive) (David *et al.* 1983; Gibert *et al.*

*For correspondence. E-mail: bcbitner@biologia.ufrj.br.

2001). In a few cases, however, some species have been able to adapt to a very different climates, really becoming cosmopolitan (David and Tsacas 1981). The latitudinal clines, mostly documented for body size, have been observed in various species not closely related including *Drosophila robusta* (Stalker and Carson 1947), *D. subobscura* (Prevosti 1995), *D. melanogaster* and *D. simulans* (Capy *et al*. 1993; Gibert *et al*. 2004), *D. kikkawai* (Karan *et al*. 1998) and *Zaprionus indianus* (Karan *et al*. 2000; David *et al*. 2006b). Moreover, body size is a highly-plastic trait, which generally decreases with increasing temperature (Angilletta and Dunham 2003; Angilletta *et al*. 2004), the so called temperature-size rule. So, under a warm tropical climate, the size of the wild-living flies (which is the expected target of natural selection), will be reduced due to two independent processes: a small genetic size due to the cline, and a small phenotypic size due to plasticity (James *et al*. 1997). The parallelism between plasticity and genetic variation is an indirect argument in favour of a better fitness of small individuals in a warm environment (Atkinson and Sibly 1997).

We are aware of two species: *D. melanogaster* and *D. simulans*, in which parallel-clinal variations were observed for body size and body size plasticity (Morin *et al*. 1999). In both cases, the tropical population was found to be smaller than the temperate one under laboratory conditions (the usual cline), but also its reaction norm was shifted towards a high temperature side, suggesting a thermal adaptation of plasticity.

In the present work, we studied several components of the wing morphology, analysed their reaction norms and compared two climatic races of the drosophilid *Z. indianus*. We investigated an African population from São Tomé Island, which lives under the stable thermal conditions of the equator and is remarkable by its small size, in agreement with a latitudinal cline (David *et al*. 2006b). The other is a Brazilian population from Rio de Janeiro (see Loh and Bitner-Mathé 2005; Machado *et al*. 2005), recently introduced from Africa. Because of their recent introduction, all South American populations, in spite of a broad latitudinal range, from Uruguay to the equator do not show any latitudinal variation. Their precise origin is not known, but their big size suggests, a country of fairly high latitude, for example Sénégal or South Africa (see David *et al*. 2006b, for discussion).

We found that the two populations differ not only by their size, as already known, but also by the reaction norms of the size, and shape traits across temperatures. In almost all cases, the significant differences between the two populations were mainly observed at the lower developmental temperatures.

## Materials and methods

Population investigated

Wild-living adults were collected in Rio de Janeiro (southeast area of Brazil; 22°53′S, 43°13′W) in December 2000 and in São Tomé Island (west of the African continent; 0°12′N, 6°39′E) in January 2001. They were brought to the laboratory and the females were isolated to establish isofemale lines. Each line was then kept in the laboratory as a mass culture for two to three generations at 20°C–22°C.

Eleven lines were available for the Rio de Janeiro population, and 11 for São Tomé. From each line, 10 females and 10 males were randomly taken to make the parents of the experimental flies. After a few days, each group was allowed to oviposit for 4–6 h in a culture vial containing killed yeast, high nutrient food (David and Clavel 1965). Such food is convenient for preventing crowding and minimizing the vial effect. The procedure was repeated five times, and each set of culture vials were transferred, in a randomized way, to incubators regulated (±0.1°C) at 17, 21, 25, 28 and 31°C. Multiple-temperature treatments are required to get a proper idea of the shape of a reaction norm. The use of very few treatments (e.g. two or even three temperatures) might be misleading, suggesting general patterns that would be only indicative of differences between extremes of the reaction norm, and potentially masking the most interesting changes (see Debat *et al*. 2003, for an example). After emergence, adults from each line were transferred to fresh food, aged for a few days and then fixed in a preservative liquid (70% alcohol, acetic acid, glycerol, 8:1:1 ratio).

Wing measurements

The left wing of each individual was mounted on a microscope slide and magnified 60× for measurements. The wing of *Drosophila* can be geometrically described by adjusting an ellipse to its contour (Klaczko and Bitner-Mathé 1990; Bitner-Mathé and Klaczko 1999a,b) (figure 1). The contour picture permits the definition of an overall ellipse with its major axis (*a*) and short axis (*b*).
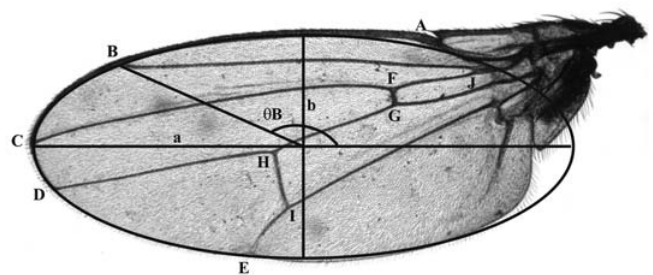
Figure 1. Adjusted ellipse of *Zaprionus indianus* wing blade. Lower case letters *a* and *b* represent the long and short axis of the ellipse, respectively. Capital letters represent the landmarks corresponding to insertion points of the wing veins. The angle θB illustrates the position of landmark B with respect to the major axis of the ellipse. Other angles are calculated in a similar way, for all landmarks.

The size and shape information is provided by: *a*, akin to wing length; *b*, akin to wing width; $SI = \sqrt{(ab)}$, overall size and $SH = b/a$, overall shape. From the centre of the

ellipse, a line is drawn towards each landmark and the angle is calculated (in radians) between the major axis and the line (see figure 1 for angle θB). Ten angles, represented by the Greek letter θ followed by landmark's letter, indicate the positions of 10 wing-vein landmarks. Although the size and form of the wing might be biologically correlated, the ellipse adjustment allows *SI*, *SH* and angle estimates to be geometrically independent, expressing different ellipse properties. For example, the ellipse area can change without changing its outline shape, and vice-versa.

Data analysis

The *Z. indianus* is a tropical species, which is easily reared at 31°C (Karan *et al*. 1999; Araripe *et al*. 2004) but is sensitive to cold. From our initial protocol, it was expected that 10 females and 10 males could be investigated from each line and temperature. It turned out that in some vials developmental accidents led to insufficient fly number resulting in an irregular data set so that we could not use an isofemale line balanced design (David *et al*. 2005). Thus, for each line and temperature, all available adults were measured, up to a maximum of 10 flies of each sex for each line. Further, the data for each temperature and population were pooled into a single sample. The numbers of investigated individuals and of available lines are given in table 1, for a grand total of 1686 wings.

Data analyses were carried out using standard statistical methods described by Sokal and Rohlf (1995). Statistical analyses were implemented on SYSTAT version 10 (Wilkinson 1997). Analysis of covariance (ANCOVA) was used to test the homogeneity of trait × temperature regressions, considering the locality and sex as effects. The magnitude of

plasticity was estimated by the coefficient of variation (CV), considering the trait means at each of the five temperatures.

Table 1. Number of flies: females ($n_{\female}$) and males ($n_{\male}$) investigated at various temperatures in the two geographic populations. The number of available lines *N* is also given.

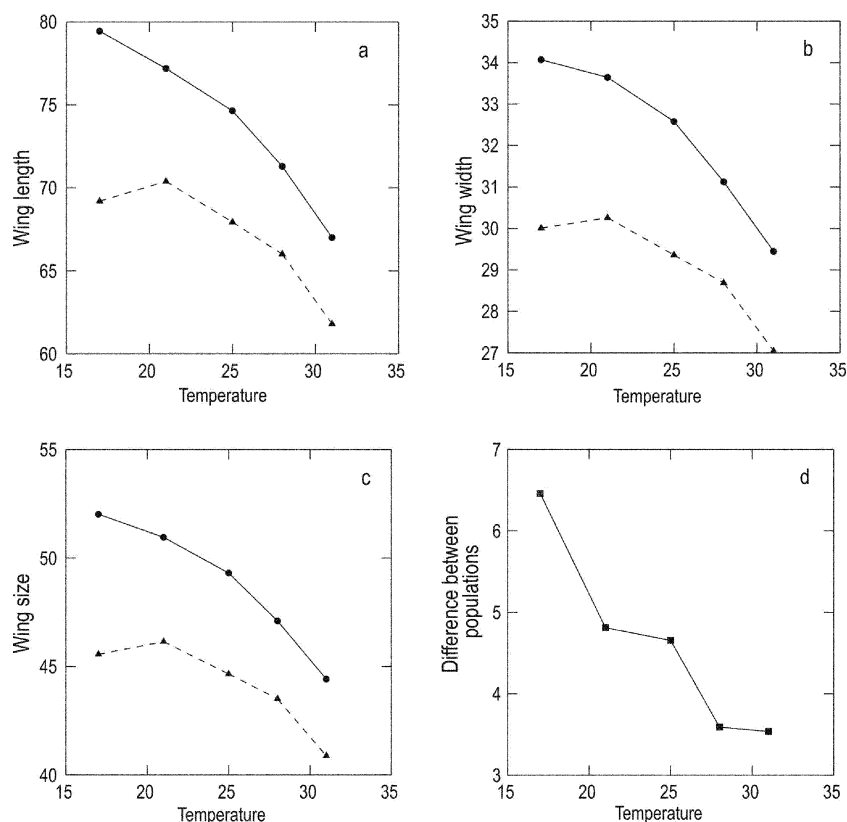| Temperature (°C)	Rio de Janeiro			São Tomé		
	$n_{\female}$	$n_{\male}$	*N*	$n_{\female}$	$n_{\male}$	*N*
17	26	24	4	99	98	10
21	106	104	11	99	99	10
25	78	79	8	110	98	11
28	83	78	9	97	101	11
31	63	71	9	87	86	10

Results

Wing size

Figure 2 shows the variation of mean values according to growth temperature for the wing length, width and overall wing size (*SI*). Overall mean values and the CV of the three wing size estimates (*a*, *b* and *SI*) are given in table 2. Flies from Rio are on average bigger than São Tomé ones. Moreover, they are also more plastic (CV means, 6.05 for Rio de Janeiro and 4.68 for São Tomé; $t = 6.1$, $df = 4$; $P < 0.004$).

Table 3 shows the ANCOVA results. Significant effects were detected for population, temperature and population × temperature interaction, indicating that Rio de Janeiro and São Tomé populations differ in their wing size mean and also in their reaction norms across temperature. Moreover, in each case, the difference between the two populations is more pronounced at low temperature than at high temperature. This phenomenon is illustrated in figure 2,d which

Table 2. Mean values (averaged over temperature and sex) and CVs of the various wing traits measured. *a*, Wing length (mm × 60); *b*, wing width (mm × 60); *SI*, overall wing size (= $\sqrt{(ab)}$mm × 60); *SH*, overall wing shape (*b/a*) and the 10 angles (radians) that determine the wing-vein positions.

	General mean ± S.E.		CV (%)	
	Rio de Janeiro	São Tomé	Rio de Janeiro	São Tomé
a	73.39 ± 0.51	67.06 ± 0.32	6.33	4.96
b	32.00 ± 0.20	29.07 ± 0.13	5.78	4.44
SI	48.46 ± 0.32	44.15 ± 0.19	6.02	4.62
SH	0.4364 ± 0.0007	0.4338 ± 0.0008	1.45	1.91
θA	0.686 ± 0.032	0.665 ± 0.002	4.20	3.73
θB	2.692 ± 0.002	2.680 ± 0.002	0.77	0.73
θC	3.140 ± 0.001	3.143 ± 0.008	0.34	0.27
θD	3.317 ± 0.001	3.325 ± 0.001	0.28	0.31
θE	4.188 ± 0.004	4.200 ± 0.005	0.95	1.25
θF	0.591 ± 0.004	0.544 ± 0.003	6.14	5.12
θG	0.440 ± 0.029	0.404 ± 0.002	5.94	5.06
θH	3.356 ± 0.004	3.406 ± 0.003	1.17	0.99
θI	4.279 ± 0.008	4.309 ± 0.008	1.66	1.90
θJ	0.415 ± 0.016	0.407 ± 0.001	3.60	3.71

The CV is calculated using the means of the five different growth temperatures.

Figure 2. Mean values of wing-size traits (mm × 60) according to growth temperature in the population of Rio de Janeiro (circles) and São Tomé (triangles). a, wing length (*a*); b, wing width (*b*); c, wing size (*SI*); d, linear decrease of the wing size difference between Rio de Janeiro and São Tomé with increasing temperature. Data from both sexes were very similar and were pooled for clarity.

shows, that the difference in *SI* between the two populations decreases almost linearly with growth temperature.

Since the reaction norms of the three-size traits (figure 2) were all concave with a maximum value, we calculated the coordinates of the maximum, using a nonlinear quadratic regression procedure (see David *et al*. 2006a, for technical details). For each available line, two parameters were calculated, the maximum value (*MV*) which compares the average size for the two populations, and the temperature of maximum value (*TMV*) which refers to plasticity. For fitting the polynomial regression of a line, missing values were completed by using the mean of other available lines of the same population at same temperature. In some cases, calculation provided unlikely values and the corresponding line was discarded.

Results are summarized in table 4. Lower-maximum values in São Tomé illustrate the smaller genetic size of that population. Besides, its higher temperature of maximum value indicates an adaptation of plasticity to a warmer environment.

Overall wing shape

Overall wing shape was analysed by considering the *SH* index, which is the ratio of the width to the length of the ellipse:

a higher value indicates a more rounded wing. We found a general trend which is an increase of *SH* with increasing temperature, except for the São Tomé population at 17°C (figure 3). In other words, when the temperature increases, there is a relative broadening of the wing. The effect of temperature on *SH* is significant, but ANCOVA (table 3) did not detect any significant effect of the sex, population or any other interaction.

The proportion of *SH* variation that might be explained by the variation in *SI* (allometric variation) was estimated through the regression of ln(*SH*) on ln(*SI*). The *SI* accounted for 16% of *SH* variation in Rio de Janeiro (ANOVA: $SS_{regression} = 0.002732$; $SS_{residual} = 0.014351$; $df = 1, 80$; $F = 15.2$; $P < 0.001$) and 8.5% in São Tomé (ANOVA: $SS_{regression} = 0.003173$; $SS_{residual} = 0.034281$; $df = 1, 102$; $F = 9.44$; $P < 0.01$). These values were not significantly different (ANCOVA: *F* for population × ln(*SI*) interaction = 0.276; $df = 1, 186$; $P = 0.6$).

Wing vein variation

The average values of the 10 angles (table 2) are remarkably variable; four of them are less than one and, not surprisingly,

Table 3. Analysis on covariance (ANCOVA) testing the effects of population (P), sex (S), temperature (T) and their interactions for a, wing length; b, wing width; SI, (overall wing size $= \sqrt{(ab)}$); SH, (overall wing shape $= b/a$) and the 10 angles that determine the wing-vein positions.

Factor	df	a	b	SI	$SH \times 10^{-4}$	$\theta A \times 10^{-4}$	$\theta B \times 10^{-4}$	$\theta C \times 10^{-4}$	$\theta D \times 10^{-4}$	$\theta E \times 10^{-4}$	$\theta F \times 10^{-4}$	$\theta G \times 10^{-4}$	$\theta H \times 10^{-4}$	$\theta I \times 10^{-4}$	$\theta J \times 10^{-4}$
P	1	417***	74***	176***	0.1	9.1	30.8**	5.5	27.6***	236***	40.7**	44.5**	318***	466***	28.2***
S	1	1.7	0.4	0.8	0.0	13.6	2.2	0.6	0.3	36.5	11.7	5.2	0.2	103	3.9
T	1	2066***	319***	814***	7.6***	430***	0.3	10.4**	7.2*	142***	729***	226***	79.5*	455***	30.6***
P × S	1	1.8	0.9	1.4	0.2	7.3	1.8	0.3	0.2	1.3	10.8	5.4	29.0	1.9	2.8
P × T	1	148***	23***	59***	0.3	0.4	18.7*	4.3	19.9***	213***	0.9	6.0	144***	364***	20.0**
S × T	1	10.2	2.0	4.5	0.0	16.5	0.8	0.2	0.0	39.9	7.8	3.8	1.5	75.7	6.0
P × S × T	1	2.3	0.9	1.5	0.1	4.7	2.5	1.6	0.1	1.4	0.6	2.7	22.5	2.2	2.0
Error	178	3.9	0.6	1.4	0.5	4.6	4.0	0.8	0.8	12.1	5.9	4.0	12.3	28.9	1.9

The table gives the mean squares (MS) with significance levels (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Bonferroni correction for the number of simultaneous comparisons gives $\alpha = 0.0036$.
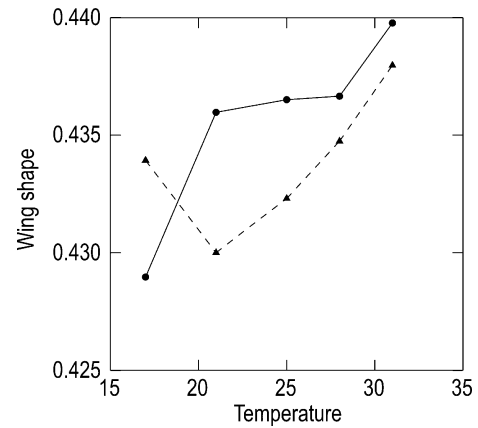
Figure 3. Mean values of overall wing shape (*SH*) in response to growth temperature in the populations of Rio de Janeiro (circles) and São Tomé (triangles). Data from both sexes were very similar and were pooled for clarity.

exhibit high CV. Such differences among angles are easily understood if we consider the definition of each of them (see figure 1): low values characterize the four basal landmarks.

The population reaction norms for each angle is illustrated in figure 4. The ANCOVA (table 3) showed a significant population difference for all angles, except θA and θC. A linear component due to temperature was also found in all cases, except θB. A significant population × temperature interaction was observed in six angles, revealling different reaction norm shapes in the two populations. Angle θB, for which there is no temperature linear component, exhibits in fact a quadratic norm, which is apparently the same for the two populations. For the other angles, there is either an increase or a decrease with growth temperature. In all cases, the greatest difference between the two populations, when significant, is always observed at low temperatures.

Discussion

The *Z. indianus* is a tropical African species which has extended its range first to the East (India) and more recently to South and North America. Within Africa, which harbours ancestral populations, clinal variations of size have been observed (David *et al.* 2006b) but we did not know which part of Africa might be the real cradle of the species. A recent discovery of two sibling species of *Z. indianus*, called *Z. africanus* and *Z. gabonensis* (Yassin *et al.* 2007) in equatorial Africa now suggests that *Z. indianus* itself was primarily, an equatorial species. The population of São Tomé investigated here is an equatorial island population; it is likely that, because of its domestic status, it has been introduced by man from nearby continental Africa, for example from Gabon, and is thus a genuine representative of equatorial Africa.

The Rio de Janeiro population, with its big size, probably has its origin at a higher latitude in Africa, possibly over

Table 4. Means and standard errors of maximum value (MV) and temperature of maximum value (TMV) for wing length (*a*), wing width (*b*), overall size (*SI*). *N* is the number of lines for which the polynomial regression was fitted considering the sum of sexes. A *t*-test was used to compare the population means.

	$MV \pm$ S.E. (mm $\times$ 60)			$TMV \pm$ S.E. (°C)		
	Rio de Janeiro	São Tomé	*t*	Rio de Janeiro	São Tomé	*t*
Wing length	79.9 ± 0.4 (*N* = 9)	70.4 ± 0.3 (*N* = 21)	19.4***	14.9 ± 0.6 (*N* = 9)	19.5 ± 0.6 (*N* = 21)	4.6**
Wing width	34.2 ± 0.1 (*N* = 16)	30.3 ± 0.1 (*N* = 20)	25.3***	16.9 ± 0.6 (*N* = 16)	19.3 ± 0.6 (*N* = 20)	3.1**
Overall size	52.2 ± 0.1 (*N* = 13)	46.2 ± 0.1 (*N* = 20)	27.4***	15.9 ± 0.6 (*N* = 13)	19.8 ± 0.4 (*N* = 20)	5.7***

For fitting the polynomial regression of a line, missing values were extrapolated by using the mean of other available lines of the same population at the same temperature. In some cases, calculation provided unlikely values and the corresponding line was discarded. $**P < 0.01$; $***P < 0.001$.

30°S. We can, therefore, reasonably conclude that the two populations correspond to the extremes of a latitudinal cline. Choosing extreme populations is a convenient way to study adaptive genetic differences among geographic populations, and such a strategy has been used earlier, for example, by Morin *et al*. (1999) and Gilchrist and Partridge (2001).

Body size variation was analysed using three wing measures and provided two straightforward conclusions. First, and as already known (David *et al*. 2006b), the Rio de Janeiro population exhibited a bigger size. Second, the reaction norms of the two populations were clearly different and the temperature of maximum value (information on the wing plasticity) was much higher in the São Tomé population living in a warmer environment. To our knowledge, this is the third case for which a genetic difference in plasticity is found between geographic populations, the two other cases being *D. melanogaster* and *D. simulans* (Morin *et al*. 1999). Similar trends of greater amplitude were observed when different species were compared (David *et al*. 1997, 2004). Differences in plastic responses between *D. simulans* and *D. buzzatii*, and among geographical populations within these species were also observed by Bubliy and Loeschcke (2005), however, the range of temperature analysed was not large enough to describe the shape of the reaction norms.

A general evolutionary rule may thus be suggested: for size-related traits that exhibit a concave reaction norm, the maximum value is observed at a higher temperature in heat adapted populations or species. Conversely, as is presumably the case in *Zaprionus* and *Drosophila*, an adaptation to a colder climate decreases the temperature of maximum value.

As compared to wing size, which is defined in about the same way in different laboratories, wing shape is far more difficult to define and analyse, because several techniques should be used. As a rule, all these methods are designed to get rid, at least in part, of size variations, and are generally expressed as ratios. Our elongation index provided straightforward information, valid for both popula-

tions, which is an increase of the width/length ratio with increasing temperature: wings are more elongated at low temperatures. In the literature, some information is found on the wing length/width ratio, generally called wing-aspect ratio. Results similar to those found in *Zaprionus* i.e., an elongation of the wing at low developmental temperature, were obtained in *D. melanogaster* (Azevedo *et al*. 1998), *D. buzzatii* (Loeschcke *et al*. 2000), and *D. simulans* (Matta and Bitner-Mathé 2004). Genetic studies, comparing wing shape variations across latitude are more controversial. Several papers have described an increase in the wing-aspect ratio with latitude (Stalker and Carson 1947; Sokoloff 1965; Azevedo *et al*. 1998; Hoffmann and Shirriffs 2002); while other works did not detect this pattern of latitudinal variations (Van't Land *et al*. 1999; Loeschcke *et al*. 2000). Gilchrist *et al*. (2000) examined the wing-aspect ratio in *D. melanogaster* and found latitudinal differences for only one of the three continents.

Concerning the variation on the wing-vein position, we found that different angles could exhibit very different reaction norms, illustrating a relative independence between different landmarks and the complexity of these wing trait variations in response to change in developmental temperature. Similar results were provided using Procrustes analysis to characterize the phenotypic plasticity of *D. simulans* wings (Debat *et al*. 2003). Moreover, we observed that the second wing vein (landmark B) shifts towards a more proximal position at both extremes of the thermal range. This was also observed for *D. simulans* (Debat *et al*. 2003; Matta and Bitner-Mathé 2004). Debat *et al*. (2003) suggested that stress, rather than temperature *per se*, is the key developmental factor affecting this pattern of wing shape variation.

Another interesting observation, made on *Z. indianus*, was that the reaction norms of the wing trait differed between the two populations and that these differences were usually greater at low temperatures of development. *Drosophila* wings are composed by two major compartments, the anterior and posterior. These compartments reflect the allocation
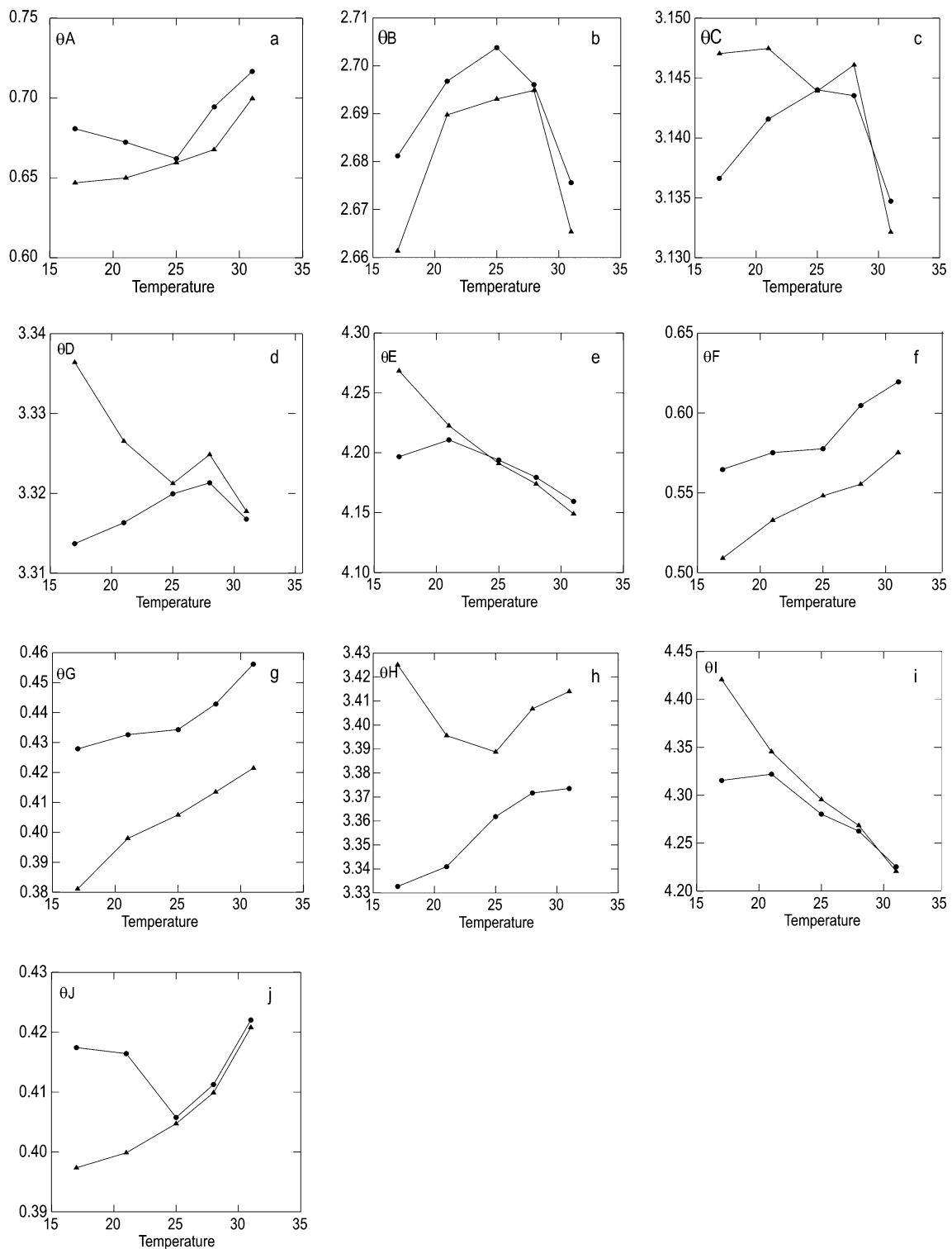
Figure 4. Mean values of 10 angles (radians) corresponding to the position of wing landmarks (figure 1) in response to growth temperature: in the population of Rio de Janeiro (circles) and São Tomé (triangles). Data from both sexes were very similar and were pooled for clarity.

of cells in early development, and their boundary lies right above the fourth longitudinal vein (Garcia-Bellido 1973). Studies have shown that there might be genes with more gen-

eral control of the wing vein placement throughout the wing blade, while other genes might control the positioning of the veins within a single compartment, or have vein-specific ac-

tion (Thompson 1975; Thompson and Woodruff 1982; Weber 1992). Genes involved in wing patterning may be affected by thermal regimes during the development and the phenotypic plasticity itself could have an adaptive nature (Starmer and Wolf 1989; Gavrilets and Scheiner 1993a,b; Pigliucci 2001). Our results show that two different genotypes may exhibit very similar or very different patterns of response to temperature variation, depending on the range of temperature analysed, corroborating the idea that comparision between populations and between species should be made across a broad range of environments including both optimal and stressful ones.

References

Andrewartha H. and Birch L. C. 1954 *The distribution and abundance of animals*. University of Chicago Press, Chicago.

Angilletta M. J. and Dunham A. E. 2003 The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. *Am. Nat.* **162**, 332–342.

Angilletta M. J., Steury T. S. and Sears M. W. 2004 Temperature growth rate and body size in ectotherms: fitting pieces of a life history puzzle. *Integr. Comp. Biol.* **44**, 498–509.

Araripe L. O., Klaczko L. B., Moreteau B. and David J. R. 2004 Male sterility thresholds in a tropical cosmopolitan drosophilid, *Zaprionus indianus. J. Therm. Biol.* **29**, 73–80.

Atkinson D. and Sibly R. M. 1997 Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends Ecol. Evol.* **12**, 235–239.

Azevedo R. B. R., James A. C., McCabe J. and Partridge L. 1998 Latitudinal variation of wing: thorax size ratio and wing-aspect ratio in *Drosophila melanogaster. Evolution* **52**, 1353–1362.

Bitner-Mathé B. C. and Klaczko L. B. 1999a Plasticity of *Drosophila melanogaster* wing morphology: effects of sex, temperature and density. *Genetica* **105**, 203–210.

Bitner-Mathé B. C. and Klaczko L. B. 1999b Heritability, phenotypic and genetic correlations of size and shape of *Drosophila mediopunctata* wings. *Heredity* **83**, 688–696.

Bubliy O. A. and Loeschcke V. 2005 Variation of life-history and morphometrical traits in *Drosophila buzzatii* and *Drosophila simulans* collected along an altitudinal gradient from a Canary island. *Biol. J. Linn. Soc.* **84**, 119–136.

Capy P., Pla E. and David J. R. 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.

Cossins A. and Bowler K. 1987 *Temperature biology of animals*. Chapman and Hall, London.

David J. R. and Clavel M. F. 1965 Interaction entre le genotype et le milieu d'elevage. Consequences sur les caracteristiques du development de la *Drosophile. Bull. Biol. Fr. Belg.* **99**, 369–378.

David J. R. and Tsacas L. 1981 Cosmopolitan, subcosmopolitan and widespread species: different strategies within the Drosophilid family (Diptera). *C. R. Soc. Biogr.* **57**, 11–26.

David J. R., Allemand R., Van Herrewege J. and Cohet Y. 1983 Ecophysiology: abiotic factors. In *Genetics and biology of Drosophila* (ed. M. Ashburner, H. L. Carson and J. N. Thompson), pp. 105–170. Academic Press, New York.

David J. R., Gibert P., Gravot E., Pétavy G., Morin J. P., Karan D. and Moreteau B. 1997 Phenotypic plasticity and developmental temperature in *Drosophila*: analysis and significance of reaction norms of morphometrical traits. *J. Therm. Biol.* **22**, 441–451.

David J. R., Gibert P. and Moreteau B. 2004 Evolution of reaction norms. In *Phenotypic plasticity: functional and conceptual approaches* (ed. T. J. DeWitt and S. M. Scheiner), pp. 50–63. Oxford University Press, New York.

David J. R., Gibert P., Legout H., Pétavy G., Capy P. and Moreteau B. 2005 Isofemale lines in *Drosophila*: an empirical approach to quantitative trait analysis in natural populations. *Heredity* **94**, 3–12.

David J. R., Legout H. and Moreteau B. 2006a Phenotypic plasticity of body size in a temperate population of *Drosophila melanogaster*: when the temperature-size rule does not apply. *J. Genet.* **85**, 9–23.

David J. R., Araripe L. O., Bitner-Mathé B. C., Capy P., Goñi B., Klaczko L. B. *et al*. 2006b Quantitative trait analysis and geographic variability of natural populations of *Zaprionus indianus*, a recent invader in Brazil. *Heredity* **96**, 53–62.

Debat V., Begin M., Legout H. and David J. R. 2003 Allometric and non-allometric components of *Drosophila* wing shape respond differently to developmental temperature. *Evolution* **57**, 2773–2784.

DeWitt T. J. and Scheiner S. M. 2004 *Phenotypic plasticity: functional and conceptual approaches*. Oxford University Press, New York.

Garcia-Bellido A., Ripoll P. and Morata G. 1973 Developmental compartmentalisation of the wing disc of *Drosophila. Nat. Biol.* **245**, 251–253.

Gavrilets S. and Scheiner S. M. 1993a The genetics of phenotypic plasticity. 5. Evolution of reaction norm shape. *J. Evol. Biol.* **6**, 31–48.

Gavrilets S. and Scheiner S. M. 1993b The genetics of phenotypic plasticity. 6. Theoretical predictions for directional selection. *J. Evol. Biol.* **6**, 49–68.

Gibert P., Moreteau B., Pétavy G., Karan D. and David J. R. 2001 Chill-coma tolerance, a major climatic adaptation among *Drosophila* species. *Evolution* **55**, 1063–1068.

Gibert P., Capy P., Imasheva A., Moreteau B., Morin J. P., Pétavy G. and David J. R. 2004 Comparative analysis of morphological traits among *Drosophila melanogaster* and *D. simulans*: genetic variability, clines and phenotypic plasticity. *Genetica* **120**, 165–179.

Gilchrist A. S., Azevedo R. B., Partridge L. and O'Higgins P. 2000 Adaptation and constraint in the evolution of *Drosophila melanogaster* wing shape. *Evol. Dev.* **2**, 114–124.

Gilchrist A. S. and Partridge L. 2001 The contrasting genetic architecture of wing size and shape in *Drosophila melanogaster. Heredity* **86**, 144–152.

Hoffmann A. A. and Parsons P. A. 1997 *Extreme environmental change and evolution*. Cambridge University Press, London.

Hoffmann A. A. and Shirriffs J. 2002 Geographic variation of wing shape in *Drosophila serrata. Evolution* **56**, 1068–1073.

James A. C., Azevedo R. B. R. and Partridge L. 1997 Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics* **146**, 881–890.

Karan D., Munjal A. K., Gibert P., Moreteau B., Parkash R. and David J. R. 1998 Latitudinal clines for morphometrical traits in *Drosophila kikkawai*: a study of natural populations from the Indian subcontinent. *Genet. Res.* **71**, 31–38.

Karan D., Moreteau B. and David J. R. 1999 Growth temperature and reaction norms of morphometrical traits in a tropical drosophilid: *Zaprionus indianus. Heredity* **83**, 398–407.

Karan D., Dubey S., Moreteau B., Parkash R. and David J. R. 2000 Geographical clines for quantitative traits in natural populations of a tropical drosophilid: *Zaprionus indianus. Genetica* **108**, 91–100.

Klaczko L. B. and Bitner-Mathé B. C. 1990 On the edge of a wing. *Nature* **346**, 321.

Leather S., Walker K. and Bale J. 1993 *The ecology of insects overwintering*. Cambridge University Press, Cambridge.

Loeschcke V., Bundgaard J. and Barker J. S. F. 2000 Variation in body size and life history traits in *Drosophila aldrichi* and *D. buzzatii* from a latitudinal cline in eastern Australia. *Heredity* **85**, 423–433.

Loh R. and Bitner-Mathé B. C. 2005 Variability of wing size and shape in three populations of a recent Brazilian invader, *Zaprionus indianus* (Diptera: Drosophilidae), from different habitats. *Genetica* **125**, 271–281.

Machado T., Solé-Cava A. and David J. R. and Bitner-Mathé B. C. 2005 Allozyme variability in an invasive drosophilid, *Zaprionus indianus* (Diptera Drosophilidae): comparison of a recently introduced Brazilian population with old world populations. *Ann. Soc. Entomol. Fr.* **41**, 7–13.

Matta B. P. and Bitner-Mathé B. C. 2004 Genetic architecture of wing morphology in *Drosophila simulans* and an analysis of temperature effects on genetic parameter estimates. *Heredity* **93**, 330–341.

Morin J. P., Moreteau B., Pétavy G. and David J. R. 1999 Divergence of reaction norms of size characters between tropical and temperate populations of *Drosophila melanogaster* and *D. simulans. J. Evol. Biol.* **12**, 329–339.

Pigliucci M. 1996 How organisms respond to environmental changes: from phenotypes to molecules (and vice-versa). *Trend Ecol. Evol.* **11**, 168–173.

Pigliucci M. 2001 *Phenotypic plasticity: beyond nature and nurture*. Johns Hopkins University Press, Baltimore.

Precht H., Christophersen J., Hensel H. and Larcher W. 1973 *Temperature and life*. Springer, Berlin.

Prevosti A. 1955 Geographical variability in quantitative traits in populations of *D. subobscura. Cold Spr. Harb. Symp. Quant. Biol.* **20**, 294–299.

Schlichting C. D. and Pigliucci M. 1993 Control of phenotypic plasticity via regulatory genes. *Am. Nat.* **142**, 366–370.

Sokal R. and Rohlf F. J. 1995 *Biometry*, 3rd edition. W. H. Freeman and Company, New York.

Sokoloff A. 1965 Geographic variation of quantitative characters in populations of *Drosophila pseudoobscura. Evolution* **19**, 300–310.

Stalker H. D. and Carson H. L. 1947 Morphological variation in natural populations of *Drosophila robusta* Sturtevant. *Evolution* **1**, 237–248.

Starmer W. T. and Wolf L. L. 1989 Causes of variation in wing loading among *Drosophila* species. *Biol. J. Linn. Soc.* **37**, 247–261.

Thompson J. N. 1975 Quantitative variation and gene number. *Nature* **258**, 665–668.

Thompson J. N. and Woodruff R. C. 1982 Polygenic analysis of pattern-formation interdependence among veins in the same compartment of the *Drosophila* wing. *Genetica* **60**, 71–76.

Van't Land J., Van Putten P., Zwaan B., Kanping A. and Van Delden W. 1999 Latitudinal variation in wild populations of *Drosophila melanogaster*: heritabilities and reaction norms. *J. Evol. Biol.* **12**, 222–232.

Weber K. E. 1992 How small are the smallest selectable domains of form. *Genetics* **130**, 345–353.

Wilkinson L. 1997 *Systat for Windows*, version 7.0. SPSS, Chicago.

Yassin A., Capy P., Madi-Ravazzi L., Ogereau D. and David J. R. 2007 DNA barcode discovers two cryptic species and two geographical radiations in the invasive drosophilid *Zaprionus indianus. Mol. Ecolo. Res.* **8**, 491–501.