Thermal plasticity of wing size and shape in *Drosophila melanogaster*, *D. simulans* and their hybrids

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ABSTRACT: Populations of *Drosophila melanogaster*, as well as of other *Drosophila* species, show body size differences according to their geographic origin. Thermal selection is considered to be the most likely cause explaining these differences. We investigated wing size, wing shape and their relationship in 3 different geographic populations of *D. melanogaster*, 1 population of *D. simulans*, and their interspecific hybrids grown at 18, 21, 25 and 28°C. The aim was to explore how the past adaptive history of 2 related species, or populations of the same species, modulates the plastic response to the environment. The wing size plasticity of hybrids between the temperate *D. simulans* (Bologna) and the 2 tropical *D. melanogaster* populations (Belém and Rio de Janeiro) was higher than that of parental species, probably as a side effect of a decanalized and compromised development at higher temperatures. The wing size plasticity of Bologna hybrids was the same as the parents, suggesting that the 2 species are subjected to the same plasticity selection. Wing shape was typical of each species, population and temperature. Shape differences increased in hybrids at higher temperatures as a consequence of developmental perturbation. The allometric relationship between size and shape changed among temperatures and among species, suggesting that the wing development is differently regulated in the 2 species and can be altered by natural selection. The allometry changed between populations of different geographic origin of *D. melanogaster*, but was similar in the 2 species that shared the same selective environment. Two species would have been subjected to the same plasticity selection, so the breakdown of the plastic response is avoided in hybrids. As a whole, these data suggest that thermal plasticity is a trait under selection and that similar mechanisms are at work in different species.

KEY WORDS: *Drosophila* · Hybrids · Development · Evolutionary constraints · Phenotypic plasticity

1. INTRODUCTION

The global surface temperature has increased by an unprecedented 0.8°C over the past 100 yr and by 0.2°C per decade in the last 30 yr (Hansen et al. 2006, Serreze 2010). At the moment, predictions about the ability of organisms to respond to climate change by plastic responses and/or by adaptation (evolutionary responses) are the most challenging and exciting fields in evolutionary biology. Phenotypic traits affecting life history (i.e. all the events that characterise the whole life cycle of an organism; Peters 1983) are grouped together and called life history traits. Body size is a pivotal trait, as other life history traits such as metabolic rate, energy requirements and reproductive success are directly affected by body size (Schmidt-Nielsen 1983, Calder 1984).

In *Drosophila* there is a strong correlation between body size (often measured as wing size) and fitness (Roff

1992). Laboratory experiments have shown that there is a positive relationship between body size and fertility, longevity, mating success and lifespan (Robertson 1957, Tantawy & Vetukhiv 1960, Partridge & Farquhar 1983, Wilkinson 1987) in non-stressful environments.

Drosophila melanogaster, as well as other *Drosophila* species, has been extensively used to describe the pattern of geographical variation in body size. Comparison among natural populations showed that body size increases with latitude and altitude and, most importantly, that part of the observed variation is of genetic origin (Reed & Reed 1948 Partridge & French 1996). Long-term selections for temperature in the laboratory resulted in divergent body sizes, with more larger flies at colder temperatures as in the wild (Anderson 1973, Cavicchi et al. 1985, 1989, Partridge et al. 1994).

Plasticity—the ability of an organism to react to internal or external environmental inputs with a change in form, state, movement or rate of activity (West-Eberhard 2003)—is becoming more important in conservation biology because selective pressures acting on populations are much higher than they were in the pre-industrialized world. Species must adjust to an environment that changes continuously, often towards extreme conditions. Studies on phenotypic plasticity demonstrate that the plastic responses are heritable and subjected to selection, and that the heritability and effectiveness of selection depend on the range of environmental conditions where selection occurs (Stearns 1989, Scheiner & Lyman 1991, Scheiner 1993, de Jong 1995, Price et al. 2003). Since both thermal plasticity and thermal selection act in the same direction on *Drosophila* body size, it is reasonable to suppose that the plastic response is adaptive (West-Eberhard 2005, Trotta et al. 2006).

Previous studies of wing shape have revealed significant interpopulation differentiation within *Drosophila* species (e.g. Imasheva et al. 1995, Haas & Tolley 1998, Griffiths, Schiffer & Hoffmann 2005), as well as phenotypic plasticity across developmental temperatures and thermal evolution in the laboratory (Cavicchi et al. 1985, 1991, Santos et al. 2004). Wing shape can be analyzed using geometric morphometric approaches, which precisely separate morphological variation (i.e. variation in form) into size (a 1-dimensional trait) and shape (an inherently multidimensional space), which can be evaluated by using the Procrustes method to obtain coordinates of shape by removing the effects of size (Klingenberg et al. 2002). Allometry is defined as shape changes associated with size changes (Penin et al. 2002) and has been shown to occur in *Drosophila* wings.

In the present study, we investigated wing size and shape of 3 different populations of *Drosophila melanogaster*, 1 population of *D. simulans*, and their interspecific hybrids. Interspecific hybrids often show reduced

vitality and/or fertility. The difference between closely related species may depend on either minor changes at many genes (Charlesworth et al. 1982) or on a wide genetic (and developmental) reorganization (Carson & Templeton 1984).

Different kinds of interaction may cause hybrid disorder: aminoacidic differences may produce non-functional proteins (Rawson & Burton 2002); some post-transcriptional pathways may be destroyed (Braidotti & Barlow 1997); or some genes may be deregulated and therefore under- or over-expressed (Michalak & Noor 2003, Ranz et al. 2004). Interspecific *Drosophila* hybrids show different kinds of morphological abnormalities which are strongly influenced by rearing temperature (Sturtevant 1920). Hybrids often have atrophic gonads (Lachaise et al. 1986) and, as a consequence, they are sterile. The abnormalities reflect deleterious genetic interactions principally due to independent and uncoordinated changes in the 2 parental genetic pools accumulated in the absence of genetic flow (Dobzhansky 1936, Muller 1942) and to differences in gene expression (Orr et al. 1997, Sucena et al. 2003, Ranz et al. 2004).

Drosophila melanogaster and *D. simulans* are morphologically similar, cosmopolitan and human commensal species, but they differ in traits such as courtship behaviour, genital morphology, ecophysiology, DNA and protein polymorphisms (Capy & Gibert 2004), and they rarely cross in the wild. In the laboratory, sterile unisexual hybrid progeny may be obtained, the sex being that of the *D. melanogaster* parent.

With the aim to provide insight into the evolution of the norm of reaction, we explored how the past adaptive history of 2 related species, as well of different geographic populations of the same species, affects the plastic response to the environment (temperature). In particular, the response of interspecific hybrids would reveal if the same adaptive strategies are at work in 2 different species. We also investigated wing allometry (i.e. the relationship between wing size and shape) and its plasticity in both parental species and hybrids, as it reflects growth developmental patterns and the interactions between species (or populations) and local climatic conditions.

2. MATERIALS AND METHODS

2.1. Populations

Three populations of *Drosophila melanogaster* (2 tropical and 1 temperate), and 1 temperate population of *D. simulans* were used. The temperate populations came from Bologna, Italy, collected in the same place in August 2008. Of the 2 tropical populations, one

came from Belém, Brazil (collected in 2002) and the other from Rio de Janeiro, Brazil (collected in 2004). These populations yielded about 20 generations per year in the laboratory. All populations were founded from 20 to 30 wild-collected fertilized females and kept in mass culture on standard medium at 18°C until September 2008, when the experiment started.

Though it was found that the past selective history does not change over the course of a few years (Capy et al. 1993), laboratory rearing can produce inbreeding and drift that can affect some life history traits (Matos & Avelar 2001). Nevertheless, we found that adaptive differences in some life history traits among natural populations persist in spite of laboratory adaptation or inbreeding effects; other life history traits change in only their mean values and not their plastic response over a thermal range (see Trotta et al. 2007).

2.2. Experimental procedures

To avoid maternal effects, flies of each species and population were allowed to oviposit for 1 d at a constant temperature of 25°C and at an optimal density (about 50 individuals in 60 ml vials containing 10 ml of food) for 1 generation. The emerging flies were transferred into new vials (7 females and 7 males per vial were used as parents, 3 vials for each population) with food (vials were smeared with abundant yeast) and maintained until adult emergence at different constant temperatures: 18, 21, 25 and 28°C.

Drosophila melanogaster virgin females of a given population were also crossed with *D. simulans* males, at a ratio of 20:30; all the offspring of these crosses consisted of hybrid females. To obtain hybrid progeny, 3 vials for each cross were also maintained at each experimental temperature under the same conditions as described above.

2.3. Wing measurements

Since all hybrid progenies were female, only females were considered in all groups. About 30 females per population and temperature were randomly chosen; the right wing of each fly was dissected, dehydrated in ethanol and mounted on glass in lactic acid/ethanol (6:5). All wings were photographed using a digital camera connected to a dissecting microscope, and photos were stored directly on a computer. From each wing, 11 landmark coordinates were recorded (Fig. 1) using ImageJ software.

2.4. Procrustes superimposition

After the landmark coordinates were recorded, all wing configurations were superimposed onto a consensus configuration (the overall mean configuration) using the Procrustes generalized least square procedure (Rohlf & Slice 1990, Rohlf & Marcus 1993, Bookstein 1991, Dryden & Mardia 1998). The new coordinates, or Procrustes coordinates, are amenable to standard multivariate analyses; since there are 4 eigenvalues that are zero in the Procrustes fit, generalised inverses or principal component scores can be used.

2.5. Size variation

The size of an individual wing was estimated by the centroid size of its landmark configuration (Slice et al. 1996). The effects of species (i.e. *Drosophila melanogaster*, *D. simulans* and hybrids), temperature and population on wing size were appraised by applying a mixed model ANOVA to centroid size including species and temperature as main fixed effects, and population nested within species as a random effect.

2.6. Shape variation

We tested for shape variation among species, temperatures and populations by applying a multivariate ANOVA (MANOVA) to the scores of a principal component analysis (PCA) performed on the Procrustes coordinates, similar to the ANOVA used for the wing centroid size.

A discriminant analysis combined with a canonical variate analysis considering species and temperature as independent factors was also performed. This approach allows optimal visualization of the relative position of the different populations in the multivariate statistical space by maximizing the among-line variation.
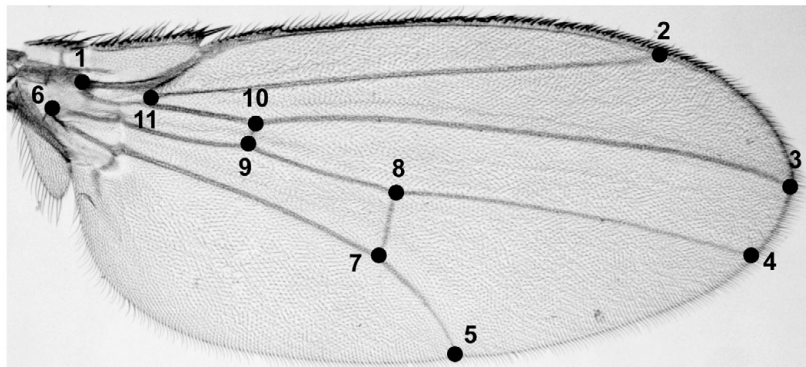

Fig. 1. *Drosophila melanogaster*. Wing landmarks used to analyze size and shape

To test for size effects on shape variation, allometric corrections were done by multivariate regression of the PC scores of the Procrustes coordinates (dependent variables) on centroid size (independent variable).

The Procrustes superimposition was done using PAST version 1.90 (Hammer et al. 2001, http://folk.uio.no/ohammer/past); all analyses in the present study were performed with R 2.9.0 (R Core Development Team 2005).

3. RESULTS

3.1. Morphological abnormalities

Interspecific hybrids at all temperatures showed morphological abnormalities, which reflects a decanalized development. Deformed individuals typically showed curly, bubbled and crumpled wings. All hybrid lines (Fig. 2) showed similar and positive temperature-dependent rates of deformations (χ^2 test, $\chi^2 = 3.4$, df = 6, p = 0.7563).

3.2. Wing size variation

Mean values of wing centroid size of females of the 3 *Drosophila melanogaster* populations, the *D. simulans* population and their hybrids at the 4 experimental temperatures are shown in Fig. 3a. As expected, the wing size of the *D. melanogaster* Bologna population was larger than in the tropical populations. Interspe-
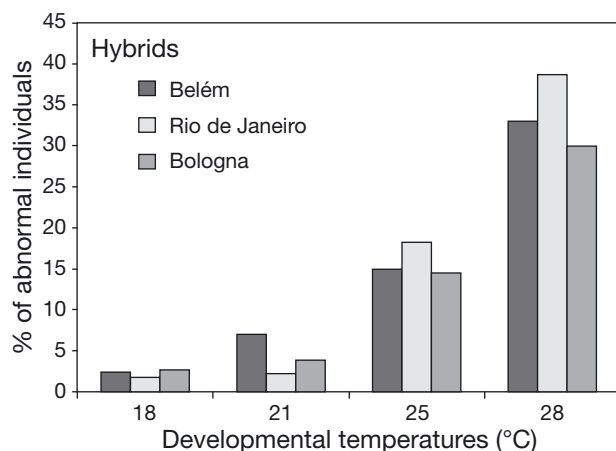

Fig. 2. *Drosophila* spp. Percentage of wing abnormalities in hybrids at the 4 different developmental temperatures: 18, 21, 25 and 28°C. Belém: hybrids between *D. melanogaster* females from Belém and *D. simulans* males from Bologna; Rio de Janeiro: hybrids between *D. melanogaster* females from Rio de Janeiro and *D. simulans* males from Bologna; Bologna: hybrids between *D. melanogaster* females from Bologna and *D. simulans* males from Bologna

cific hybrids had smaller wings than the respective *D. melanogaster* parent (except Belém hybrids at 18 and 21°C). *D. simulans* wings were always smaller than the smallest wings in the *D. melanogaster* population (Belém) and quite similar to the hybrids in size. All the groups showed a decrease in wing size when the rearing temperature increased, with significant differences between groups ($F_{12,870} = 11.8$, p < 0.001).

Fig. 3b shows the regression lines between midparent and hybrid centroid size over the 4 temperatures. The 3 hybrid lines differ in their intercepts giving evidence of a change of dominance in interspecific crosses ($F_{2,2} = 30$, p = 0.00075). Belém and Rio de Janeiro regression lines are very similar in slope. The regression coefficients are higher than 1, indicating a greater plasticity of the hybrids. Bologna hybrids behaved differently, though with a relatively low level of significance ($F_{2,6} = 4.74$, p = 0.058); the regression coefficient is close to 1, suggesting a similar response of midparents and hybrids.

3.3. Wing shape variation

The Species, Temperature and the interaction Species × Temperature effects in the MANOVA on the PC scores of the Procrustes coordinates were highly significant (p < 0.001 in all cases; Table 1). The population effect was also significant (Table 1), suggesting that wing shape also varies among *Drosophila melanogaster* populations and among hybrids with different genetic background. Separate analyses on populations or hybrids (i.e. 3 populations of *D. melanogaster* or 3 groups of hybrids) showed very high significant shape differences between populations and between hybrids (p < 0.001 in all cases, data not shown).

The discriminant analysis combined with canonical analysis provided a clear discrimination among groups. The first canonical axis, which accounted for 61% of the total variance (Fig. 4a), discriminated hybrids and parental species. Hybrids increased their wing shape divergence from parents at 25°C and even more at 28°C, probably reflecting developmental noise more evident at higher temperatures (see also Fig. 2). Interestingly, the divergence was smaller in Bologna hybrids. The second canonical axis (17% of the total variance) seemed to account only for the temperature effect (Fig. 4b).

3.4. Allometric variation

In order to test the effects of wing size on wing shape variation, an allometric correction was performed using a multivariate regression of the PC scores of the Pro-
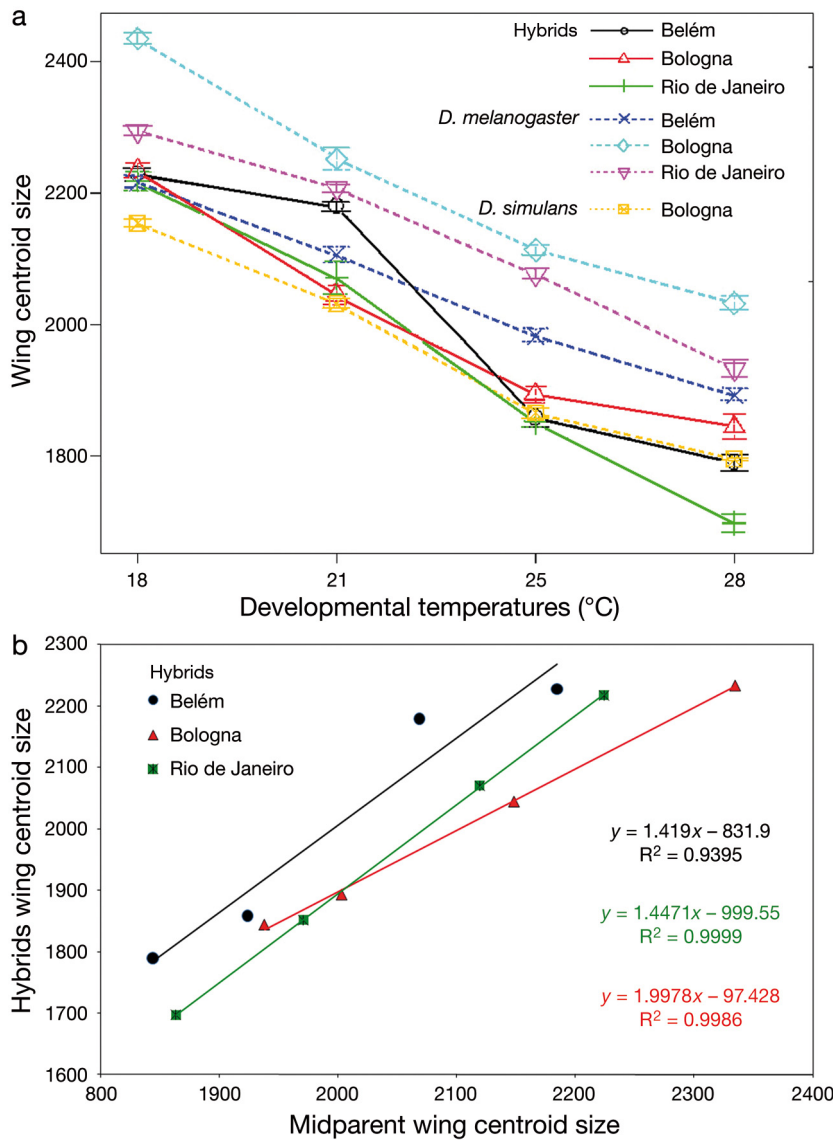
Fig. 3. *Drosophila* spp. (a) Mean wing centroid size (±SE) of the 3 *D. melanogaster* populations, *D. simulans* and their hybrids at the 4 experimental temperatures: 18, 21, 25 and 28°C. (b) Relationship between centroid size of each hybrid and the respective midparent value at the 4 experimental temperatures. See Fig. 2 for description of hybrids

crustes coordinates (dependent variables) on the wing centroid size (the independent variable). The multivariate regression coefficient of Procrustes coordinates on wing centroid size was highly significant (r = 0.842, p < 0.001), and a high amount of allometry in the *Drosophila* wing, which accounts for about 99% of the total shape variance, was found.

To test the effects of wing size on wing shape variation among species (i.e. *Drosophila melanogaster*, *D. simulans* and their hybrids) and temperature, a multivariate analysis of covariance (MANCOVA) was performed, with Species and Temperature as main effects and Population nested within Species and Temperature. All the effects in the MANCOVA were significant (p < 0.001 in all cases, data not shown). The significant differences in the relationship between wing size and shape among groups show that the developmental allometric relationship (that is, how a wing changes its shape when the size varies) changes between temperatures and, more interestingly, between species or populations. The significant differences in slopes and intercepts show that non-allometric differences among groups are present.

It is interesting to note that the relationship between wing size and shape (allometry) changes when different geographic populations of *Drosophila melanogaster* (Bologna and Rio de Janeiro), but not different species coming from the same place (i.e. *D. melanogaster* and *D. simulans* collected in Bologna; Table 2), are considered.

Table 1. Results of the MANOVA with Temperature and Species as fixed effects and Population nested within Species and within temperature performed on the PC scores of the Procrustes coordinates of hybrids, *D. melanogaster* and *D. simulans* at the 4 experimental temperatures: 18, 21, 25 and 28°C. Num: numerator; Den: denominator; Pr: probability

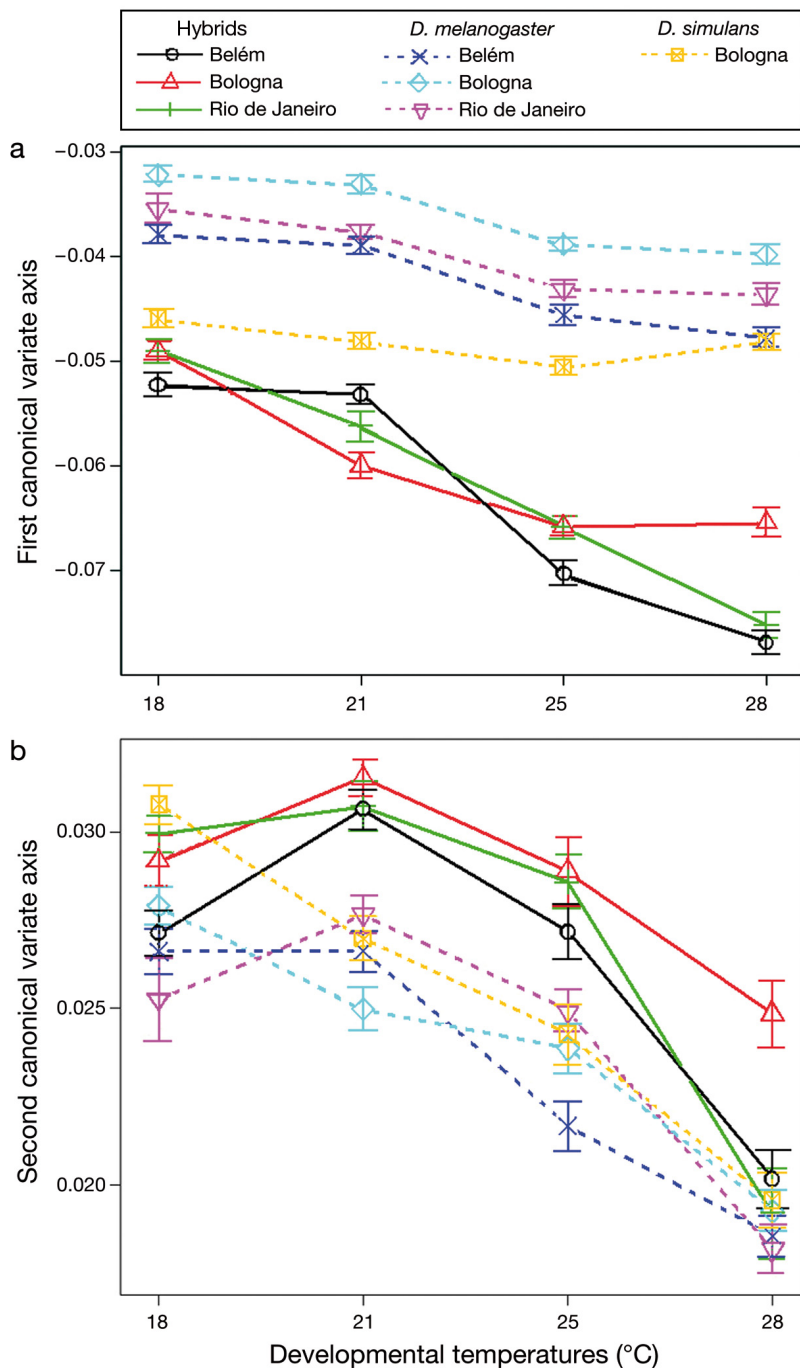| Source of variation	df	Pillai's trace	Approx F	Num df	Den df	Pr(>F)
(Intercept)	1	1.45×10^{-11}	6.5×10^{-10}	19	852	1
Species	2	1.259	76.24	38	1706	$<2 \times 10^{-16}$
Temperature	3	1.008	22.76	57	2562	$<2 \times 10^{-16}$
Species × Temperature	6	0.797	6.91	114	5142	$<2 \times 10^{-16}$
Populations within Species, within Temperature	16	1.749	5.6	304	13872	$<2 \times 10^{-16}$
Residuals	870					

Fig. 4. Mean values (±SE) of (a) the first (whole shape variation) and (b) the second canonical plan computed from the canonical variate analysis on the PC scores of the Procrustes coordinates. See Fig. 2 for description of *Drosophila* hybrids

4. DISCUSSION

We investigated the phenotypic plasticity of wing size and shape in *Drosophila melanogaster*, *D. simulans* and their interspecific hybrids. With the aim to provide insight into the evolution of the norm of reac-

tion, we explored how the past adaptive history of 2 related species, as well as of different populations of the same species, affects the plastic response to the environment.

4.1. Morphological abnormalities

The morphological abnormalities found in the hybrids were qualitatively similar to those reported by Markow & Ricker (1991) and Rego et al. (2006). Wing abnormalities exponentially increased with increasing rearing temperature, giving evidence that hybrid development is seriously compromised at higher temperatures.

It has been shown that tropical populations tolerate high temperatures better than temperate ones (Trotta et al. 2006 and references therein), hence a hybrid with half a genetic background from a tropical *Drosophila melanogaster* might be more stable at high temperatures than a hybrid with a temperate genetic background. In spite of that, no significant differences among hybrids with different genetic backgrounds (i.e. Belém hybrids, Bologna hybrids and Rio de Janeiro hybrids) were found. The results indicate that buffer mechanisms that stabilize the development against genetic perturbations (breaking of the genomic coadaptation) are independent from those involved in the adaptations to climate conditions. However, Bologna hybrids (Fig. 2) showed a slightly lower percentage of abnormalities at 28°C (even if not significant) compared to the other hybrids. Since the parents came from the same geographic location, similar environmental buffering mechanisms could be shared between species, which would account for the more stable hybrids.

4.2. Size variation

Drosophila melanogaster exhibits numerous genetic body size differences between tropical and temperate populations (David & Capy 1988, Gilchrist & Partridge 1999, David et al. 2004). The adaptive significance of

Table 2. MANCOVAs with Temperature and Populations (*Drosophila melanogaster* from Bologna and *D. melanogaster* from Rio de Janeiro) and with Temperature and Species (*D. melanogaster* and *D. simulans* from Bologna) as main effects on the global shape values covaried for wing centroid size. Num: numerator; Den: denominator; Pr: probability

| Source of variation | df | ——— *D. melanogaster* populations ——— | | | | | Bologna *D. melanogaster* and *D. simulans* | | | | |
		Pillai	Approx F	Num df	Den df	Pr(>F)	Pillai	Approx F	Num df	Den df	Pr(>F)
Size–shape regression	1	0.776	37.1	19	203	$<2.2 \times 10^{-16}$	0.863	65.4	19	198	$<2.2 \times 10^{-16}$
Among Groups (intercepts)	1	0.603	16.24	19	203	$<2.2 \times 10^{-16}$	0.753	31.72	19	198	$<2.2 \times 10^{-16}$
Among Temperatures (intercepts)	3	1.250	7.71	57	615	$<2.2 \times 10^{-16}$	1.078	5.91	57	600	$<2.2 \times 10^{-16}$
Slopes among Groups	1	0.333	5.34	19	203	1.99×10^{-10}	0.531	11.78	19	198	$<2.2 \times 10^{-16}$
Slopes among Temperatures	3	0.496	2.14	57	615	6.37×10^{-6}	0.716	3.3	57	600	2.52×10^{-13}
Among Temperatures and Groups (intercepts)	3	0.821	4.07	57	615	$<2.2 \times 10^{-16}$	0.366	1.46	57	600	0.018
Slopes among Temperatures and Groups	3	0.547	2.41	57	615	1.54×10^{-7}	0.386	1.55	57	600	0.0072
Residuals	221/216										

these differences has been well described and interpreted as a response to the ambient temperature (Anderson 1973, Cavicchi et al. 1989, Partridge et al. 1994).

We have found that differences between the populations of *Drosophila melanogaster*, considered over the whole thermal range, were not clearly transmitted to the interspecific hybrids and, in this respect, genes for size in the 2 species seemed to not cooperate in an additive way (but see David et al. 2002). Hybrids were always smaller than the midparent. Because *D. simulans* is generally smaller than *D. melanogaster* (Capy et al. 1993, Gilchrist & Partridge 1999, present study), the easiest formal interpretation is that *D. simulans* genes are somehow dominant (Moreteau et al. 1995). In other words, allelic interactions exist at the same loci. The complete data set points, however, to the more general interpretation of an F1 breakdown, probably due to deleterious epistatic gene interactions as shown, for example, in viability studies (e.g. Coyne & Orr 1998). Hybrid breakdown may be observed in F1 or F2 generations as a consequence of either an interspecific or an intraspecific cross (Dobzhansky et al. 1977), resulting in a smaller size. In conclusion, hybrid breakdown has a strong effect on body size (smaller individuals), which was particularly evident at 28°C.

4.3. Phenotypic plasticity of wing size

Apart from body size, the phenotypic plasticity of different *Drosophila melanogaster* populations seems to have also changed. *D. melanogaster* is a species of Afrotropical origin and climatic adaptation has occurred from tropical towards temperate climates (Lachaise et al. 1986). During the colonisation of temperate continents, adaptation has not only produced bigger flies, but also a shift in phenotypic plasticity: the

maximum size is observed at a lower temperature in temperate populations (Trotta et al. 2006).

In the present study, wing size plasticity of hybrids seems to be higher than parental species in Belém and Rio de Janeiro crosses. However, given the strong size reduction at higher temperatures due to hybrid breakdown, it is possible that hybrid plasticity is not an 'adaptive' plasticity, but rather a side effect of a decanalized and impaired development. It is interesting to note the wing plasticity of Bologna hybrids (Fig. 3b) is the same as that of the parental species. Once again, as the 2 species share the same selective environment, they would have been subjected to the same plasticity selection so that a breakdown of the plastic response is avoided in hybrids.

4.4. Wing shape variation

The results of the present study show that wing shape is typical of each species, population and temperature. Moreover, there are wing shape differences in hybrids at different rearing temperatures. At 18 and 21°C, wing shape plasticity in hybrids is similar to that of the 2 species, but the differences drastically increase at 25 and 28°C (Fig. 4a). It has been fully discussed that the development of hybrids is seriously compromised, especially at higher temperatures. The only possible conclusion is that, unlike body size, strong wing shape variations reflect strong developmental perturbations.

4.5. Allometric variation

The correlation between size and shape gives information on the links between the developmental machinery and the variation of a biological form. Considering shape as a univariate trait, the significant dif-

ferences in slopes and intercepts among groups found in the MANCOVA indicate that the developmental allometric relationship between size and shape (that is, how a wing changes its shape when the size varies) changes among temperatures and among species. We can conclude that wing development is differently regulated in the 2 species and can be altered by natural selection.

Interestingly, the relationship between size and shape changes between populations of the same species of different geographic origin (*Drosophila melanogaster*, Bologna and Rio de Janeiro), but only slightly between the 2 species with the same geographic origin (*D. melanogaster* and. *D. simulans* from Bologna; Table 2). These data support the hypothesis that the developmental allometric relationship between wing size and wing shape (i.e. the developmental machinery) has, under environmental selection, a similar impact on the 2 species.

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