

Clinal variation in developmental time and viability, and the response to thermal treatments in two species of *Drosophila*

GUILLERMO FOLGUERA*, SANTIAGO CEBALLOS, LUCIANA SPEZZI, JUAN JOSÉ FANARA and ESTEBAN HASSON

Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EHA Buenos Aires, Argentina

Received 18 September 2007; accepted for publication 2 January 2008

The present study first addressed the question of whether developmental time (DT) and viability (VT) vary clinally along latitudinal and altitudinal gradients in *Drosophila buzzatii*, an autochthonous specialist and the generalist invasive *Drosophila melanogaster*. Coincident and positive altitudinal clines across species and, direct and inverse latitudinal clines were observed for DT in *D. melanogaster* and *D. buzzatii*, respectively. Opposing latitudinal and altitudinal clines were detected for VT only in *D. melanogaster*. The patterns observed along altitudinal gradients prompted us to investigate whether flies living at lowland and highland environments may respond differentially to thermal treatments consisting of regimes of constant and alternating temperatures. Flies reared at higher mean temperature developed faster than at lower mean temperature in both species. By contrast, the response in VT differed greatly between species. Highland *D. melanogaster* were more viable than lowland regardless the treatment, whereas, in *D. buzzatii*, highland flies were more viable than lowland in alternating thermal regimes and the reverse was true in treatments of constant temperature. The results obtained suggest that thermal amplitude may be an important factor that should be considered in investigations of thermal adaptation. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **95**, 233–245.

ADDITIONAL KEYWORDS: thermal adaptation – thermal amplitude-clines.

INTRODUCTION

Understanding variation in life-history traits is one key to understand natural selection. Life-history traits such as survival, growth rate, fecundity, and age at maturity are considered as measurements of an organism's fitness in nature (Stearns, 1992). The expression of these phenotypic traits, as for all traits, is mediated by environmental influences. Nevertheless, life-history traits have a particularly strong influence on an organism's fitness.

Larval developmental time is a very important trait in the life history of organisms that typically mature in ephemeral resources such as rotting fruit and

necrotic cacti (Chippindale *et al.*, 1997). A faster development is expected to increase fitness in two ways, either through its positive effect on survival under conditions of larval crowding or through its putative demographic advantage for early reproduction in expanding populations. Moreover, the notion that *Drosophila* has undergone continuous directional selection for developmental speed in the wild has led to the proposal that additive genetic variance for developmental time (DT) may be exhausted in natural populations. However, there is evidence that developmental rate is not strictly maximized in *Drosophila melanogaster* (Zwaan, Bijlsma & Hoekstra, 1995; Chippindale *et al.*, 1997; Nunney & Cheung, 1997). Moreover, several studies brought up the possibility that DT and body size may be involved in a trade-off, that may provide a plausible

*Corresponding author.
E-mail: guillermofolguera@gmail.com

explanation for the maintenance of additive genetic variance for DT and, also, impose a strong constraint to traits evolution (Bonner & Horn, 1982; Gebhardt & Stearns, 1988; Neat *et al.*, 1995; Partridge *et al.*, 1999; Fernández Iriarte & Hasson, 2000; Cortese *et al.*, 2002; Bharathi *et al.*, 2004).

Latitudinal clines for body size are widely documented in *D. melanogaster* (Capy, Pla & David, 1993; Imasheva, Bubli & Lazebny, 1994; James, Azevedo & Partridge, 1995; Hallas, Schiffer & Hoffmann, 2002; Sgrò & Blows, 2004) and in other species such as *Drosophila subobscura* (Huey & Berrigan, 2001). In addition, it has been shown that body size evolves in the direction of larger size in natural populations of *D. melanogaster* living at higher latitudes, and experimental populations exposed to low temperatures mimic this pattern (Crill, Huey & Gilchrist, 1996; Kari & Huey, 2000; David, Legout & Moreteau, 2006). In view of the trade-off between body size and DT, positive latitudinal (and altitudinal) clines for DT may be expected in species showing clinal variation for body size. However, the association between DT and geographic variables has been usually inferred on the basis of indirect evidence and seldom addressed in the literature (James & Partridge, 1995; James *et al.*, 1995).

Selection on juvenile stages in a complex life cycle can have numerous effects on overall fitness. From an ecological standpoint, we may expect a negative correlation between DT and preadult survival. The rationale is simple: slow developing flies would spend more time in a continuously decaying resource and exposed to predators and parasites, thus limiting survival. In opposition, there may be also internal physiological risks associated with fast development such as lower levels of developmental stability or higher rates of juvenile mortality. However, the few reports exploring the link between survival and DT yielded conflicting results; negative and positive correlations were reported in *D. melanogaster* lines selected for postponed senescence (Chippindale *et al.*, 1997) and fast development (Chippindale *et al.*, 1994). In addition, other studies reported that DT and pre-adult survival are uncoupled in *D. melanogaster* (Zwaan *et al.*, 1995) and *Drosophila buzzatii* (Fernández Iriarte & Hasson, 2000).

It is generally accepted that temperature may be one of the main selective factors accounting for latitudinal (and altitudinal) clines for body size (Atkinson & Sibly, 1997; Blackburn, Gaston & Loder, 1999). Consistent with this interpretation, experimental studies have also traditionally assessed the effect of regimes of constant temperature on fitness traits (Santos *et al.*, 2005 in *D. subobscura*). However, temperature may change in different and complex ways along latitudinal and altitudinal gradients. For

example, thermal amplitude (the difference between diurnal and nocturnal temperatures) may differ dramatically between highland and lowland environments. Moreover, thermal amplitude, which is positively correlated with altitude, has been suggested to play a selective role in the very few studies addressing its effect on life-history variation (Loeschcke *et al.*, 1997; Loeschcke, Bundgaard & Barker, 1999; Pétauy *et al.*, 2001, 2004).

In the present study, we investigated variation in DT and first-instar larva to adult viability (VT) within and among populations located along latitudinal and altitudinal gradients in an ecological specialist, the cactophilic *D. buzzatii*, and *D. melanogaster*, a generalist widespread species. Because, as we will show, comparisons between highland and lowland populations revealed consistent trends across species for DT, but not for VT, we decided to investigate whether populations of both species living at contrasting altitudes (highland versus lowland populations) may respond differentially to thermal treatments differing either in mean temperature and/or in thermal amplitude. The questions addressed were: (1) do *D. melanogaster* and *D. buzzatii* respond in similar ways to different thermal regimes in terms of DT and VT? (2) Do highland and lowland populations (of both *D. buzzatii* and *D. melanogaster*) respond differentially to thermal regimes of constant and alternating temperatures? (3) Can thermal amplitude be considered as a selective agent exerting varying selective pressures in highland and lowland populations?

MATERIAL AND METHODS

STRAINS AND SPECIES

Seven natural populations of both *D. buzzatii* and *D. melanogaster* were sampled along latitudinal and altitudinal gradients in Northwestern (February 2003) and Western Argentina (February 2004). The geographical location of the populations sampled in each species is presented in Figure 1. In addition, geographical coordinates, altitude above sea level and climatic information (thermal regimes) are given for each locality in Table 1. Correlation analysis showed that latitude and altitude were not significantly correlated in the sets of localities in which flies were collected ($r = 0.16$, $P = 0.74$ and $r = -0.39$, $P = 0.39$, in *D. melanogaster* and *D. buzzatii*, respectively). However, patterns of variation in DT and VT were investigated by means of multiple regression analyses on latitude and altitude.

Flies were collected by net sweeping on fermented banana baits and sorted by sex and species upon arrival to the laboratory. Females collected in each locality were used for the foundation of sets of iso-



Figure 1. Geographical location of the natural populations of *Drosophila melanogaster* (black symbols) and *Drosophila buzzatii* (grey symbols) sampled in the present study.

female lines (lines henceforth) by rearing the progeny of individual wild inseminated females in *Drosophila* instant medium. Lines were identified to species by the inspection of the genitalia of one progeny male. This procedure was necessary because *D. melanogaster* and *D. buzzatii* coexist with otherwise morphologically indistinguishable species (*D. simulans* and *D. koepferae*, respectively) in certain localities. Lines were maintained for five generations at low density and under a standard 12 : 12 h light/dark cycle at 25 °C.

LABORATORY EXPERIMENTS

Two sets of experiments, based on a full-sib experimental design, were performed. In the first set, which aimed to investigate patterns of variation in DT and VT along latitudinal and altitudinal gradients, twelve isofemale lines of each population and species were chosen at random from the pools of lines. For each line, 200 sexually mature flies (100 of each sex) were released in egg-collecting chambers. Medium size Petri dishes containing an egg laying medium (3 g agar, 5 mL of 3 : 1 ethanol : acetic acid 60% and 175 mL of distilled water) were used for egg collection. Petri dishes were replaced after 12 h and incubated at 25 °C until egg hatching (approximately 24 h and 36 h for *D. melanogaster* and *D. buzzatii*, respectively). Batches of 30 (in *D. melanogaster*) and 40 (in *D. buzzatii*) first-instar larvae were

transferred to vials containing *Drosophila* instant medium. For each combination of species, population and line, four replicated vials were run and incubated at 25 °C until the emergence of the last adult fly.

The second set of experiments aimed to determine whether flies living in highland and lowland localities (located at the same latitude) of both species respond differentially to various thermal treatments in terms on DT and VT. To this end, six isofemale lines were randomly chosen from the pools of lines derived from collections of *D. buzzatii* in Quilmes (highland) and Vigos (lowland), and *D. melanogaster* in Lavalle (lowland) and Uspallata (highland). Four vials (replicates) of each line were randomly assigned to each one of seven thermal treatments. In three of the treatments, temperature was maintained at 17 °C, 25 °C or 30 °C (constant temperature regimes). In the other four temperatures varied along the day, reaching a maximum during the period of light and a minimum during the night (alternating temperature regimes): 30–25 °C, 25–17 °C, 30–9 °C, and 25–9 °C (in all cases diurnal temperature is given first). Flies were exposed to the thermal treatments from the first instar larval stage until the emergence of the imagoes. Treatments were chosen according to the range of thermal regimes to which flies are exposed in the localities sampled (Table 1). Although the experimental design is not complete, because we did not include all thermal treatments of alternating temperatures

Table 1. List of the localities sampled in *Drosophila melanogaster* and *Drosophila buzzatii* along with the geographic coordinates altitude and climatic data consisting of thermal information

Species	Altitude		Temperature											
	Localities		Summer				Year				Absolute			
	Localities	Latitude	(m)	Longitude	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
<i>Drosophila melanogaster</i>	San Blas	28°25'	1061	67°06'	NA	NA	NA	NA	NA	NA	NA	NA	40.0	-5.6
	Chilecito	29°10'	1043	67°28'	34.4	20.4	27.9	13.6	39.7	13.6	27.9	13.6	39.7	-3.8
	Jachal	30°12'	1238	68°45'	31.4	16.1	24.7	8.2	40.5	8.2	24.7	8.2	40.5	-9.0
	San Juan	31°27'	671	68°31'	33.9	18.2	26.2	10.1	42.1	10.1	26.2	10.1	42.1	12.7
	Barreal	31°38'	1626	69°28'	31.4	11.4	26.2	5.7	37.1	5.7	26.2	5.7	37.1	-12.0
	Uspallata	32°35'	1915	69°22'	30.9	12.9	21.6	2.7	27.6	2.7	21.6	2.7	27.6	-16.5
<i>Drosophila buzzatii</i>	Lavalle	32°50'	647	68°28'	33.7	20.2	24.2	10.2	32.2	10.2	24.2	10.2	32.2	-2.3
	Güemes	24°38'	739	65°03'	31.6	18.4	27.9	14.4	35.0	14.4	27.9	14.4	35.0	-5.3
	Cachi	25°07'	2280	66°12'	34.9	13.5	NA	NA	29.0	NA	NA	NA	29.0	-11.3
	Quilmes	26°27'	1855	66°02'	30.0	12.9	NA	NA	31.4	NA	NA	NA	31.4	-6.7
	Vipos	26°28'	798	65°22'	30.7	19.7	25.6	14.1	38.4	14.1	25.6	14.1	38.4	0.3
	Termas	27°28'	248	64°52'	33.3	19.8	27.8	13.7	41.4	13.7	27.8	13.7	41.4	-8.0
	Catamarca	28°28'	519	65°46'	NA	NA	NA	NA	40.5	NA	NA	NA	40.5	-3.8
	San Luis	33°25'	709	66°25'	30.5	16.9	24.3	10.7	36.1	10.7	24.3	10.7	36.1	-8.1

NA, Not available.

with the same mean temperature as in all constant temperature regimes, we emphasize that our main aim was to test the hypothesis that highland and lowland populations respond differentially to temperature treatments differing in the extent of thermal amplitude.

LIFE-HISTORY TRAITS SCORED

Newly-emerged adults were recovered from the vials every 6 h. DT, measured as the time elapsed after the transfer of first instar larvae to the vials until adult emergence, and VT, as the proportion of adults emerged in each vial relative to the number of larvae initially seeded, were recorded in all lines.

STATISTICAL ANALYSIS

Multiple-regression analyses of DT and VT on latitude and altitude were employed to investigate patterns of interpopulation variation. For DT, males and females were analyzed jointly because previous analyses revealed that the sex ratio did not depart significantly from the expected 1 : 1. In addition, the sex by population interaction was not significant as determined by analysis of variance (ANOVA), which was performed for each species separately, with sex and population as fixed factors. These two pieces of evidence justified pooling sexes in regression analyses. DT and VT were regressed on latitude and altitude, using lines (12 per population) as experimental units (input data consisted of the mean values of DT and VT of each line averaged across replicates).

We also employed one-way ANOVAs with population as main effect as a more direct test to specifically address the effect of altitude on DT and VT, by means of comparisons between populations located at contrasting altitude and similar latitude in two pairs of highland-lowland populations of each species (Quilmes/Vipos and Cachi/Güemes in *D. buzzatii* and Uspallata/Lavalle and Barreal/San Juan in *D. melanogaster*).

We also conducted one-way ANOVAs for VT and DT for each population, with line (random) as the single source of variation. These tests aimed to determine the relative contribution of differences among lines to total trait variance. According to our experimental design, a significant line effect in these ANOVAs may be construed as an estimation of the contribution of genetic variation to total phenotypic variance because all lines were reared under controlled laboratory conditions (Hartl & Conner, 2004; David *et al.*, 2005). In these ANOVAs, vials were considered as experimental units.

The effects of thermal treatments on DT and VT were analyzed by means of two-way ANOVAs, with

population (highland versus lowland) and thermal treatment as main sources of variation. In these ANOVAs, lines were considered as the experimental units, a procedure that renders the tests conservative since the replicate effect may increase the error term in the variance ratio. The underlying model in these ANOVAs is:

$$y = \mu + P + T + P \times T + \epsilon,$$

where μ is the overall mean of the trait, P and T represent the effects of population and thermal treatments, respectively, and ϵ is the error.

When necessary, post-hoc comparisons were performed using Tukey's method. Prior to all statistical analysis, DT and VT data were log and angularly transformed [$\arcsin(p_i)^{1/2}$, where p_i is the percentage of survival], respectively. For all tests, we used the statistical package Statistica for Windows (version 6.0).

RESULTS

DT VARIATION AMONG POPULATIONS

DT exhibited a wide range of variation within and among populations in both species. Multiple regressions on latitude and altitude yielded significant results in both species (Fig. 2). Partial regression coefficients on latitude had different signs in *D. melanogaster* and *D. buzzatii*. On one hand, DT increased as a function of latitude in the former (Fig. 1A), whereas, in the latter, the slope was negative (Fig. 1E). By contrast, partial regressions revealed significant and positive associations between DT and altitude in both *D. melanogaster* and *D. buzzatii* (Fig. 2B, F).

However, the inverted latitudinal cline detected in *D. buzzatii* requires a critical examination of the sampling strategy because it might be an artefact resulting from particularities of the set of populations included in the survey. Although latitude and altitude are uncorrelated in the ensemble of localities studied, it is not just a detail that both highland localities (Quilmes and Cachi) are in the northern limit of the surveyed area, which could bias the results of our regression analysis. However, it is important to note that the inverted latitudinal cline was still significant ($\beta = -0.5$, $P = 6 \times 10^{-6}$) after excluding Quilmes and Cachi from the dataset.

Additional ANOVAs comparing DT between pairs of lowland and highland populations were significant in both species ($F_{1,10} = 66.4$, $P = 2.71 \cdot 10^{-14}$ in *D. buzzatii* and $F_{1,10} = 9.5$; $P = 2.1 \cdot 10^{-4}$ in *D. melanogaster*). A posteriori comparisons revealed a significantly longer DT in highland compared with lowland populations, a trend that was clear in both *D. buzzatii* (Vipos versus

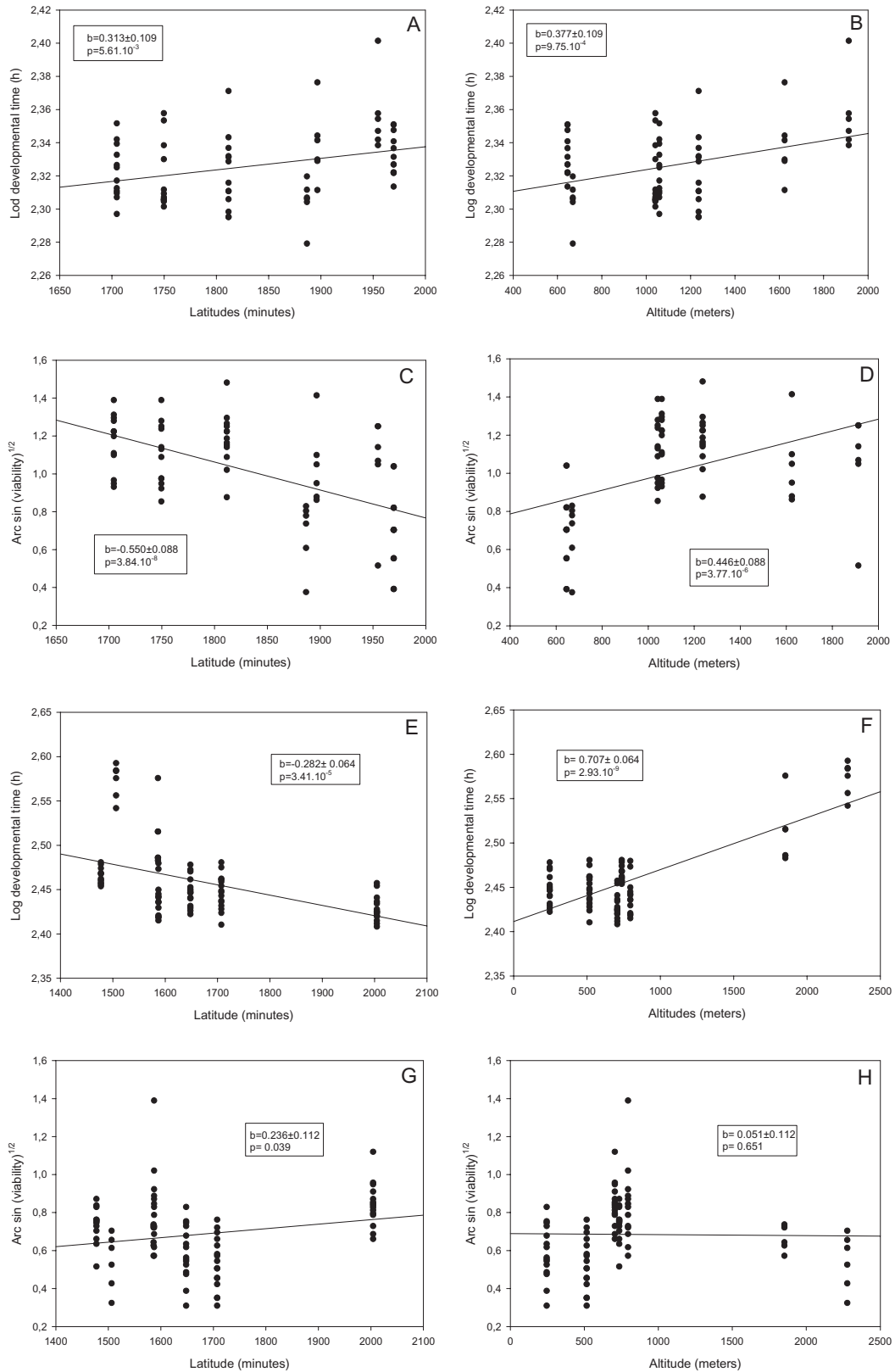


Figure 2. Altitudinal and latitudinal variation in developmental time (in hs) and viability (angularly transformed values) variation in *Drosophila melanogaster* (A–D) and *Drosophila buzzatii* (E–H). b, partial regression coefficients.

Quilmes, Tukey's test $P = 1.62 \times 10^{-4}$, and Güemes *posteriori* Cachi, Tukey's test $P = 1.32 \times 10^{-4}$, and, although less evident, in *D. melanogaster* [San Juan *posteriori* Barreal (Tukey's tests $P = 1 \times 10^{-2}$) and Lavalle *posteriori* Uspallata ($P = 4.3 \times 10^{-2}$)].

One-way ANOVAs, aimed to estimate the proportion of within population variation accounted for by differences among lines (within populations) in both species, revealed significant differences in most cases (with only one exception in *D. buzzatii*, not shown). In general, the proportion of phenotypic variance accounted for by the line component, which, according to our experimental design, may be conceived as an estimate of the genetic component of variance in DT [differences among isofemale lines (families) within populations], varied from 54–80% in *D. melanogaster* and from 37–70% in *D. buzzatii*.

VT VARIATION AMONG POPULATIONS

Differences in mean VT among populations were highly significant in both *D. buzzatii* and *D. melanogaster* ($F_{1,10} = 5.77$, $P = 2.67 \cdot 10^{-3}$ and $F_{1,10} = 6.12$, $P = 2.7 \cdot 10^{-3}$, respectively). Partial regressions of VT on latitude revealed contrasting trends in both species. On the one hand, VT was negatively and positively correlated with latitude and altitude in *D. melanogaster*, respectively (Fig. 1C, D). On the other hand, only the regression of VT on latitude was significant and positive in *D. buzzatii* (Fig. 1G–H).

As expected, comparisons between pairs of highland and lowland populations revealed subtle differences between species. Highland populations showed significantly higher mean VT than lowland populations in *D. melanogaster* (Tukey's tests: Barreal versus San Juan $P = 8.9 \times 10^{-3}$, Lavalle versus Uspallata $P = 9.3 \times 10^{-3}$), whereas in *D. buzzatii* differences between Cachi and Güemes were significant (Tukey's test: $P = 2.6 \times 10^{-3}$), and, only marginally significant between Vipos and Quilmes ($P = 7.7 \times 10^{-2}$).

Differences among lines within populations were significant in all *D. melanogaster* populations and only in three out of seven *D. buzzatii* populations. The percentage of variance accounted for by the line component varied widely from 25–67% in *D. melanogaster* and from 55–60% in *D. buzzatii*.

THE EFFECT OF THERMAL TREATMENTS ON DT AND VT

Considering the results presented so far, pointing to a consistent effect of altitude on DT and less evident on VT, we decided to investigate the effect of temperature on both DT and VT, by exposing flies to different thermal treatments from the first larval stage until the emergence of imago. To this end, we selected two

populations of each species to investigate whether highland and lowland populations (located at comparable latitudes) may respond differentially to regimes of constant and alternating temperatures. The rationale of these experiments is simple: populations living at high and low altitudes are not only exposed to different mean temperature but also experience contrasting thermal regimes along the day. Temperature reaches its maximum during the day and decreases steadily after sunset, particularly in highland localities. Indeed, climatic information, obtained from Servicio Meteorológico Nacional (Argentina) for most localities sampled in the present study showed that differences between maximum and minimum daily mean temperature were considerably larger in highland than in lowland specially during the sampling period (Table 1).

Mean values of DT and VT obtained for lowland and highland populations of *D. melanogaster* and *D. buzzatii* exposed to thermal treatments are presented in Figure 3. The first relevant feature in the ANOVAs for DT is that the population (P) by thermal treatment (T) interaction was not significant neither in *D. melanogaster* nor *D. buzzatii* (Table 2), suggesting that thermal treatments affected in similar ways highland and lowland populations. However, the patterns of variation differed between species in several features. Only the thermal treatment effect was significant in *D. melanogaster* (Table 2). In general, flies reared at higher mean temperature developed faster. Interestingly, flies reared at 17 °C had a longer mean DT than at 25–9 °C. By contrast, differences between populations and among treatments were significant in *D. buzzatii*, lowland flies developed faster than highland, regardless the treatment (Fig. 2), and flies reared at higher mean temperature developed faster than at lower mean temperature. Another interesting feature is that flies reared at 17 °C developed faster than those grown at 25–9 °C (Tukey's test: $P = 2.1 \times 10^{-4}$) (Fig. 2) at variance with the trend observed in *D. melanogaster* (Fig. 2).

The ANOVAs performed for each combination of thermal treatment and population showed a significant line effect for DT, in both *D. buzzatii* and *D. melanogaster* (Table 3), indicating that a significant proportion of phenotypic variance has a genetic basis.

Concerning VT, the two main sources of variation, population and thermal treatment were significant in *D. melanogaster* (Table 2). Highland flies were, on average, more viable than lowland flies (Fig. 2). Another important aspect of our results was the low VT in all vials in which temperature reached a maximum of 30 °C, either constantly (30–30 °C) or in regimes of alternating temperatures (30–25 °C and 30–9 °C). These results are surprising because the available evidence suggests that *D. melanogaster* can

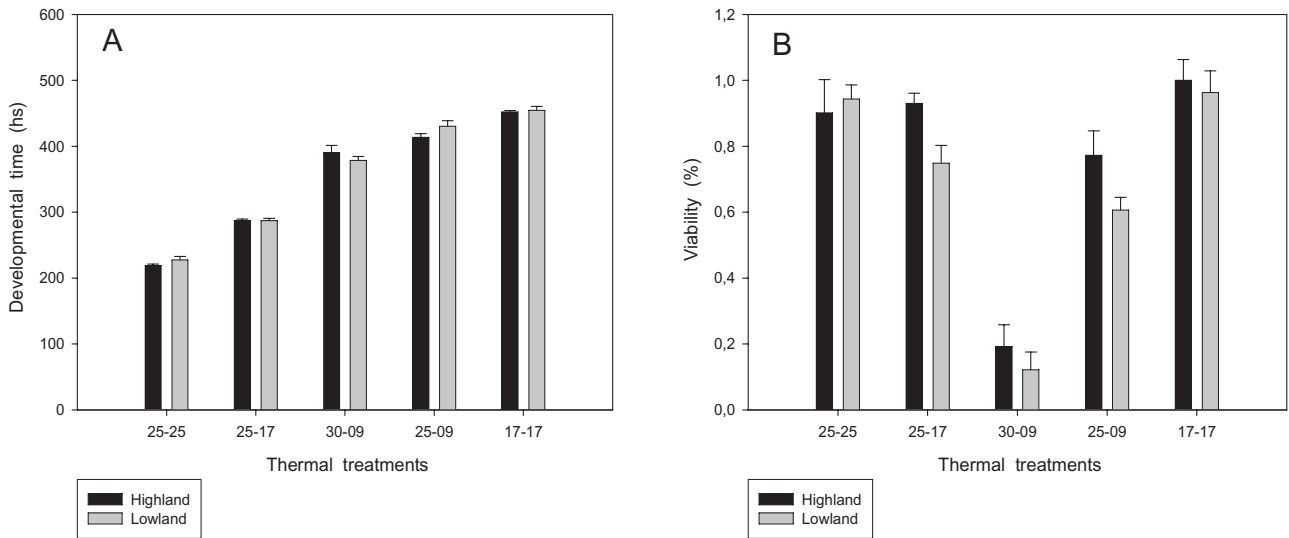
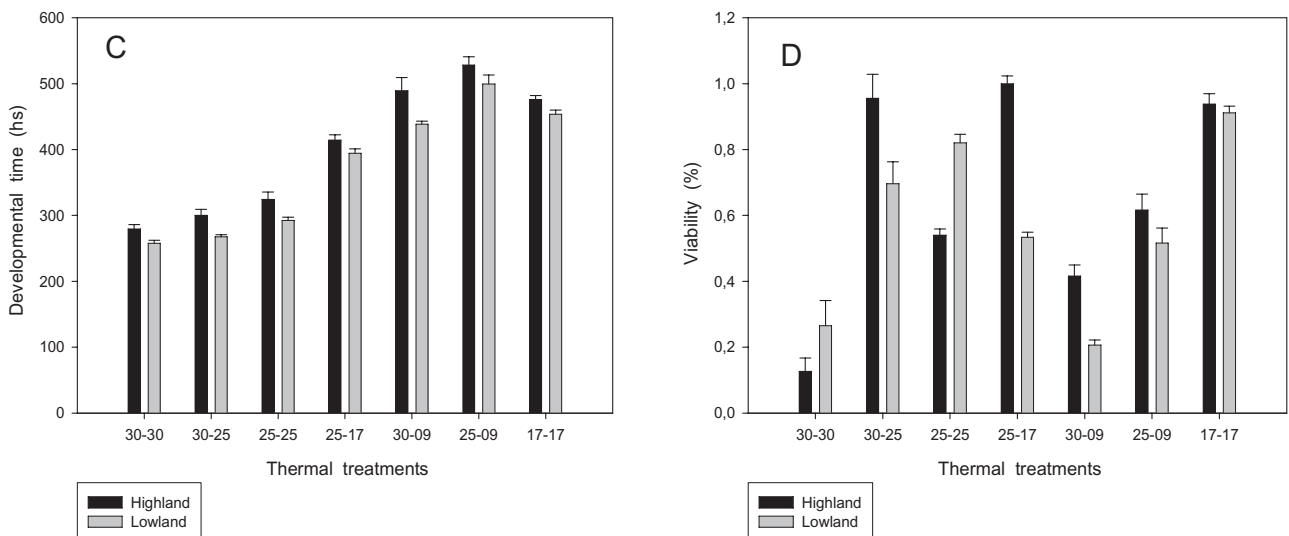
D. melanogaster*D. buzzatii*

Figure 3. Means of developmental time and viability (expressed as percent of survivors divided by the maximum value obtained in each species) in highland and lowland populations of *Drosophila melanogaster* (A, B) and, *Drosophila buzzatii* (C, D) exposed to different thermal treatments.

be successfully, although with difficulties, reared at 30 °C (Das, Mohanty & Parrida, 1994).

The ANOVA for VT revealed a significant population by thermal treatment interaction in *D. buzzatii* (Table 2), suggesting that the thermal response was not proportional across sampling localities. Interestingly, highland flies had (on average) a better performance in treatments of alternating temperatures than those from lowland and the reverse was true in

treatments of constant temperature (Fig. 2). A posteriori comparisons revealed that highland flies had a higher mean VT than lowland at 30–25 °C and 30–9 °C (Tukey's test: $P < 0.05$ in both cases), and lowland flies were more viable than highland at 25–25 °C.

Finally, the contribution of the line component to total VT variance varied sharply between species as shown by the ANOVAs performed for each combina-

Table 2. Results of the analysis of variance for developmental time and viability testing for differences between highland and lowland populations and among thermal treatments in *Drosophila melanogaster* and in *Drosophila buzzatii*

Source of variation	Developmental time				Viability			
	d.f.	SS	<i>F</i>	<i>P</i>	d.f.	SS	<i>F</i>	<i>P</i>
<i>Drosophila melanogaster</i>								
Population (P)	1	136	0.92	0.34	1	0.14	4.38	0.04
Temperature (T)	4	438 272	738.14	4.10 ⁻⁷	6	14.96	74.59	7.10 ⁻⁷
P × T	4	1 051	1.77	0.15	6	0.23	1.16	0.33
Error	45	6 680			68	2.27		
<i>Drosophila buzzatii</i>								
Population (P)	1	16 284	28.94	1.10 ⁻⁶	1	0.05	2.49	0.12
Temperature (T)	6	620 537	183.83	2.10 ⁻⁷	6	2.39	19.24	5.10 ⁻⁵
P × T	6	1 909	0.57	0.76	6	0.31	2.50	0.03
Error	64	36 006			70	1.45		

d.f., degrees of freedom; SS, sums of squares.

Table 3. Analysis of variance (ANOVA) components for developmental time and viability in highland and lowland populations of *Drosophila buzzatii* and *Drosophila melanogaster*

Species	Population	30–30	30–25	25–25	25–17	30–09	17–17	25–09
Developmental time								
<i>Drosophila melanogaster</i>	Lowland	†	†	13.94*** (51.9)‡	5.86** (32.6)	4.71* (32.6)	2.94* (32.6)	3.00* (31.9)
<i>Drosophila melanogaster</i>	Highland	†	†	17.83*** (17.7)‡	27.28*** (44.1)‡	4.12* (69.8)	9.83*** (44.1)‡	4.65** (47.1)
<i>Drosophila buzzatii</i>	Lowland	11.21** (73.1)‡	†	11.6*** (70.9)‡	9.76*** (37.5)‡	3.28* (79.9)	17.33*** (37.5)‡	17.69*** (79.2)‡
<i>Drosophila buzzatii</i>	Highland	†	14.91*** (78.9)‡	45.59*** (90.1)‡	6.05** (45.9)‡	24.37*** (86.2)‡	4.96** (61.6)‡	7.73***
Viability								
<i>Drosophila melanogaster</i>	Lowland	4.26* (74.6)	†	8.95*** (66.5)‡	6.00** (93.5)‡	11.22** (48.0)‡	4.70** (93.5)‡	3.33* (36.8)
<i>Drosophila melanogaster</i>	Highland	†	6.07* (45.0)	10.31*** (70.9)‡	2.92* (75.2)	3.79* (56.3)	2.61* (75.2)	NS
<i>Drosophila buzzatii</i>	Lowland	18.87*** (7)‡	4.09* (44.5)	NS	NS	NS	NS	7.17*** (56.4)‡
<i>Drosophila buzzatii</i>	Highland	6.25* (14.9)	6.19**	NS	NS	NS	NS	NS

In each cell, *F*-values are presented and the relative contribution of the among-line variance component in each combination of population and thermal treatment is given in parenthesis (%).

P* < 0.05, *P* < 0.01, ****P* < 0.001.

†ANOVA could not be performed.

‡Significative values after Bonferroni correction for multiple comparisons.

NS, nonsignificant.

tion of population and thermal treatment. On the one hand, the line component was significant in most treatments in *D. melanogaster*, whereas, in *D. buzzatii*, significance was restricted to just a few treatments, a trend that was robust even after Bonferroni correction for multiple comparisons (Table 3).

DISCUSSION

A large number of studies reported latitudinal clines for body size in *Drosophila* illustrating the so called Bergmann's rule (James *et al.*, 1995; Huey *et al.*, 2000). Furthermore, it is well known that general

body size is correlated with several adult fitness components (Partridge & Fowler, 1992; Tylor, Anderson & Peckarsky, 1998; Bublly & Loeschcke, 2005) and that being bigger may have the underlying cost of an extended development (Fernández Iriarte & Hasson, 2000; Cortese *et al.*, 2002). This line of reasoning leads to the prediction of positive latitudinal (and altitudinal) clines for DT in species obeying Bergmann's rule. In this sense, our results agree with the prediction, at least in *D. melanogaster* because body size covaries positively with latitude and altitude in the array of populations sampled in the present study (Folguera, 2007), in agreement with a large body of evidence (Capy *et al.*, 1993; Imasheva *et al.*, 1994; James *et al.*, 1995; Gilchrist *et al.*, 2000; Hallas *et al.*, 2002). Therefore, it may be argued that patterns of DT variation in *D. melanogaster* are consistent with the view that the evolution of DT and body size clines may be the result of a complex interaction between these two traits linked by a trade-off (Chippindale *et al.*, 1997).

The picture in *D. buzzatii* is not as simple as in *D. melanogaster*. Comparisons between highland and lowland populations revealed a consistent pattern, coincident with the trend observed in *D. melanogaster*. However, the sign of the latitudinal cline was opposite to the predicted on the basis of body size clines observed in *D. buzzatii* (Fanara, Hasson & Rodríguez, 1997; Folguera, 2007).

In general, latitudinal and altitudinal clines in *D. melanogaster* and the altitudinal cline in *D. buzzatii* are consistent with the prediction that cold climate may lead to longer DT and larger body size. Furthermore, the trends observed in nature are consistent with our own experimental results, illustrating that highland flies and flies exposed to cooler rearing temperatures have an extended development (see below).

Bergmann's rule has called the attention of biologist for more than a century, and temperature variation has been invoked as the causal factor in endotherms (Blanckenhorn *et al.*, 2006). However, the causes of Bergmann's rule in ectotherms may require an alternative explanation because small ectotherms, similar to many insects, can acclimate almost instantly to thermal conditions. Moreover, cases of converse Bergmann's rule, which are thought to be mediated by season length rather than temperature, have been reported in insects (Blanckenhorn, 1997; Mousseau, 1997; Chown & Klok, 2003; Blanckenhorn & Demont, 2004). Under this view, shorter growing seasons at higher latitudes would progressively limit development leading, indirectly, to a decrease in body size, suggesting that the primary target of selection may be developmental rate rather than body size. However, the latter is not a likely explanation for the reversed latitudinal cline observed in *D. buzzatii*

because the decrease in DT in southern populations is not accompanied by a concomitant decrease in mean body size. In addition, studies investigating the relationship between body size and polymorphic inversions in *D. buzzatii* (Fernández Iriarte & Hasson, 2000), provide indirect evidence in favor of a direct relationship between body size and latitude. Body size increasing arrangements covary positively with latitude and altitude, while size decreasing arrangements exhibit the opposite trend (Rodríguez *et al.*, 2000).

For VT, clines were clear in *D. melanogaster* whereas the trends were weaker in *D. buzzatii*. These results are surprising because the statistical power to detect clines should be higher in *D. buzzatii* as, in the latter, populations were sampled over a much larger latitudinal gradient (11°) than in *D. melanogaster* (4°). Indeed, the fact that population samples were collected at varying altitudes along the latitudinal gradient may impose a limit to the power of the regression analysis. Thus, we cannot rule out that the absence of a latitudinal trend in survival in *D. buzzatii* may be a consequence of the sampling strategy, in view of the strong differential response of highland and lowland populations to the thermal regimes detected in this species.

A necessary condition for considering clines as an adaptive response of the gene pool to environmental variation is that trait variation should be heritable. In this sense, there is ample evidence indicating that DT and VT variation have a genetic basis in both *D. melanogaster* (Van Delden & Kamping, 1991; Prasad *et al.*, 2000) and *D. buzzatii* (Fernández Iriarte & Hasson, 2000; Fanara *et al.*, 2006), suggesting that the observed clines may be the outcome of natural selection.

In general, latitudinal and/or altitudinal clines have been explained as an adaptive response to varying thermal regimes. However, the patterns of variation observed in natural populations suggest that mean temperature may only provide a partial explanation. Thus, we decided to evaluate the effect of several thermal regimes in which temperature was either maintained constant or alternate between day and night during development on lowland and highland populations because thermal amplitude differs sharply between highland and lowland localities.

Interestingly, these experiments revealed important differences between species in their responses to thermal regimes. In general, the cosmopolitan *D. melanogaster* does not appear to have evolved differences in response of either DT or VT in highland versus lowland populations, whereas, in the specialist *D. buzzatii*, highland and lowland populations responded quite differently to treatments of alternating and constant temperature, particularly for VT.

Differences between highland and lowland populations in DT were similar across treatments and between species, which appeared to have responded to mean temperature rather than thermal amplitude, though a weak effect of thermal amplitude was apparent in both species. By contrast, differences between species were evident when the responses to thermal treatments were evaluated in terms of survival. On one hand, highland flies were more viable than lowland and the magnitude of the interpopulation differences did not vary across treatments in *D. melanogaster*. On the other hand, highland flies were more viable than lowland in treatments of alternating temperatures and the trend was reversed in treatments of constant temperature in *D. buzzatii*, suggesting that this species, an inhabitant of arid and semiarid regions, has evolved adaptations that increase the chances of survival under the stringent conditions imposed by thermal fluctuations. Moreover, these ecologically-relevant responses to thermal regimes also point out that thermal fluctuations may be an important selective factor in natural populations of *D. buzzatii*.

Furthermore, our estimates of genetic variation for VT were consistently lower in *D. buzzatii* (Table 3) than in *D. melanogaster*. From a theoretical standpoint, traits that directly affect fitness (like survival) are expected to exhaust additive genetic variance under directional selection, whereas traits with distant and/or indirect effect on fitness are expected to have greater heritabilities (Mousseau & Roff, 1987; Roff & Mousseau, 1987). These expectations are fulfilled for VT and DT in *D. buzzatii*. Highland and lowland populations responded quite differently to thermal treatments, suggesting that alternative alleles have become fixed in genes responsive to thermal variation in nature. Thus, the specific responses of highland and lowland populations may be construed as plastic responses of otherwise different genotypes.

Finally, it is interesting to note that the pattern of responses to thermal treatments and the associated genetic component of VT in *D. melanogaster* differed sharply from *D. buzzatii*. However, these differences might be accounted for by the recent history of the former in South America and its population dynamics in the localities sampled. In effect, collecting records in Lavalle revealed that flies can be found all year round probably because of the availability of different types of fruits along the year. However, populations in areas characterized by a marked seasonality, like Uspallata, are probably reestablished from refugia or human mediated transport via agricultural products. If the first alternative is true flies should be able to overwinter and endowed with physiological mechanisms such as the ability to enter diapause, that may

facilitate overwintering. Indeed, a recent study in North American *D. melanogaster* has revealed a correlation between diapause incidence and latitude (Schmidt *et al.*, 2005), and it would be worthwhile to examine the relationship between diapause incidence and altitude in the populations sampled in the present study.

ACKNOWLEDGEMENTS

The authors wish to thank A. Fontdevila, J. S. F. Barker, J. David, an anonymous reviewer, and J. A. Allen for comments, suggestions and advice on earlier versions of this paper. We thank I. Camillioni and M. Rusticucci for their help and advice with the climatological data, which was obtained from the European Project CLARIS (A Europe South America Networks for Climate Change Assessment and Inspect Studies) of the Sixth PCRDT of the European Union 2004–2007. G.F. is recipient of a postgraduate scholarship of Universidad de Buenos Aires. E.H. and J.J.F. are members of Carrera del Investigador Científico of CONICET. This work was supported by Universidad de Buenos Aires, ANPCyT and CONICET grants.

REFERENCES

- Atkinson D, Sibly RM. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology and Evolution* **12**: 235–239.
- Bharathi NS, Prasad NG, Shakarad M, Joshi A. 2004. Correlates of sexual dimorphism for dry weight and development time in five species of *Drosophila*. *Journal of Zoology* **264**: 87–95.
- Blackburn TM, Gaston KJ, Loder N. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and Distributions* **5**: 165–174.
- Blanckenhorn WU. 1997. Altitudinal life history variation in the dung flies *Scathophaga stercoraria* and *Sepsis cympea*. *Oecologia* **109**: 342–352.
- Blanckenhorn WU, Demont M. 2004. Bergmann and Converse Bergmann latitudinal clines in Arthropods: two ends of a continuum? *Integrative and Comparative Biology* **44**: 413–424.
- Blanckenhorn WU, Stillwell RC, Young KA, Fox CW, Ashton KG. 2006. When Rensch meets Bergmann: does sexual size dimorphism change systematically with latitude. *Evolution* **60**: 2004–2011.
- Bonner JT, Horn HS. 1982. Selection for size, shape and development timing. In: Bonner JT, ed. *Evolution and development*. New York, NY: Springer-Verlag, 259–276.
- Bubliy OA, Loeschke V. 2005. Variation of life-history and morphometrical traits in *Drosophila buzzatii* and *Drosophila simulans* collected along an altitudinal gradient from a Canary island. *Biological Journal of the Linnean Society* **84**: 119–136.

- Capy P, Pla E, David JR. 1993.** Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans*. I. Geographic variations. *Genetics, Selection, Evolution* **25**: 517–536.
- Chippindale AK, Alipaz JA, Chen HW, Rose MR. 1997.** Experimental evolution of accelerated development in *Drosophila*. 1 Developmental speed and larval survival. *Evolution* **51**: 1536–1551.
- Chippindale AK, Hoang DT, Service PM, Rose MR. 1994.** The evolution of development in *Drosophila melanogaster* selected for postponed senescence. *Evolution* **48**: 1880–1899.
- Chown SL, Klok CJ. 2003.** Altitudinal body size clines: latitudinal effects associated with changing seasonality. *Ecography* **26**: 445–455.
- Cortese MD, Norry FM, Piccinali R, Hasson E. 2002.** Direct and correlated responses to artificial selection on developmental time and wing length in *Drosophila buzzatii*. *Evolution* **56**: 2541–2547.
- Crill WD, Huey RB, Gilchrist GW. 1996.** Within and between generation effects of temperature on the morphology and physiology of *Drosophila melanogaster*. *Evolution* **50**: 1205–1218.
- Das A, Mohanty S, Parrida BB. 1994.** Abdominal pigmentation and growth temperature in Indian *Drosophila melanogaster*: evidence for genotype-environment interaction. *Journal of Biosciences* **19**: 267–275.
- David JR, Gibert P, Legout H, Petavy G, Capy P, Moreteau B. 2005.** Isofemale lines in *Drosophila*: an empirical approach to quantitative traits analysis in natural populations. *Heredity* **94**: 3–12.
- David JR, Legout H, Moreteau B. 2006.** Phenotypic plasticity of body size in a temperate population of *Drosophila melanogaster*: when the temperature-size rule does not apply. *Journal of Genetics* **85**: 9–23.
- Fanara JJ, Folguera G, Iriarte PF, Mensch J, Hasson E. 2006.** Genotype by environment interactions and development time in populations of cactophilic *Drosophila*. *Journal of Evolutionary Biology* **19**: 900–908.
- Fanara JJ, Hasson E, Rodríguez C. 1997.** The effect of polymorphic inversions on body size in two natural populations of *Drosophila buzzatii* from Argentina. *Heredity* **126**: 233–237.
- Fernández Iriarte P, Hasson E. 2000.** The role of the use of different host plants in the maintenance of the inversion polymorphism in the cactophilic *Drosophila buzzatii*. *Evolution* **54**: 1295–1302.
- Folguera G. 2007.** Analysis of the effects of the temperature over physiological characters and life history traits in species of *Drosophila*. PhD Thesis, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.
- Gebhardt MD, Stearns SC. 1988.** Reaction norms for developmental time and weight at eclosion in *Drosophila mercatorum*. *Journal of Evolutionary Biology* **1**: 335–354.
- Gilchrist AS, Azevedo RBR, Partridge L, O'Higgins PO. 2000.** Adaptation and constraint in the evolution of *Drosophila melanogaster* wing shape. *Evolution & Development* **2**: 114–124.
- Hallas R, Schiffer M, Hoffmann AA. 2002.** Clinal variation in *Drosophila serrata* for stress resistance and body size. *Genetical Research* **79**: 141–148.
- Hartl DL, Conner JK. 2004.** *A primer of ecological genetics*. Sunderland, MA: Sinauer Associates.
- Huey RB, Berrigan D. 2001.** Temperature, demography and ectotherm fitness. *The American Naturalist* **158**: 204–210.
- Huey RB, Gilchrist GW, Carlson ML, Berrigan D, Serra L. 2000.** Rapid evolution of a geographic cline in size in an introduced fly. *Science* **287**: 308–309.
- Imasheva AG, Bublil OA, Lazebny OE. 1994.** Variation in wing length in Eurasian populations of *Drosophila melanogaster*. *Heredity* **72**: 508–514.
- James AC, Azevedo RBR, Partridge L. 1995.** Cellular basis and developmental timing in size cline of *Drosophila melanogaster*. *Genetics* **140**: 659–666.
- James AC, Partridge L. 1995.** Thermal evolution of rate of larval development in *Drosophila melanogaster* in laboratory and field populations. *Journal of Evolutionary Biology* **8**: 315–330.
- Kari JS, Huey RB. 2000.** Size and seasonal temperature in free-ranging *Drosophila subobscura*. *Journal of Thermal Biology* **25**: 267–272.
- Loeschcke V, Bundgaard J, Barker JSF. 1999.** Reaction norms and genetic parameters at different temperatures for thorax and wing size traits in *Drosophila aldrichi* and *D. buzzatii*. *Journal of Evolutionary Biology* **12**: 605–623.
- Loeschcke V, Krebs RA, Dahlgard J, Michalak P. 1997.** High-temperature stress and the evolution of thermal resistance in *Drosophila*. In: Bijlsma R, Loeschcke Y, eds. *Environmental stress, adaptation and evolution*. Basel: Birkhäuser, 175–192.
- Mousseau TA. 1997.** Ectotherms follow the converse Bergmann's rule. *Evolution* **51**: 630–632.
- Mousseau TA, Roff DA. 1987.** Natural selection and the heritability of fitness components. *Heredity* **59**: 181–198.
- Neat F, Fowler K, French V, Partridge L. 1995.** Thermal evolution of growth efficiency in *Drosophila melanogaster*. *Proceedings of the Royal Society of London Series B, Biological Sciences* **260**: 73–78.
- Nunney L, Cheung W. 1997.** The effect of temperature on body size and fecundity in female *Drosophila melanogaster*: evidence for adaptive plasticity. *Evolution* **51**: 1529–1535.
- Partridge L, Fowler K. 1992.** Direct and correlated responses to selection for age at reproduction in *Drosophila melanogaster*. *Evolution* **46**: 76–91.
- Partridge L, Langelan R, Fowler K, Boszwaan KF, French V. 1999.** Correlated responses to selection on body size in *Drosophila melanogaster*. *Genetical Research* **74**: 43–54.
- Prasad NG, Shakarad M, Gohil VM, Sheeba V, Rajamani M, Joshi A. 2000.** Evolution of reduced preadult viability and larval growth rate in laboratory populations of *Drosophila melanogaster* selected for shorter development time. *Genetical Research* **76**: 249–259.
- Pétavy G, David JR, Debat V, Gibert P, Moreteau B. 2004.** Specific effects of cycling stressful temperatures upon phenotypic and genetic variability of size traits in *Drosophila melanogaster*. *Evolutionary Ecology Research* **6**: 873–890.

- Pétavy G, David JR, Gibert P, Moreteau B. 2001.** Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. *Journal of Thermal Biology* **26**: 29–39.
- Rodríguez C, Piccinali R, Levy E, Hasson E. 2000.** Contrasting population genetic structures using allozymes and the inversion polymorphism in *Drosophila buzzatii*. *Journal of Evolutionary Biology* **13**: 976–984.
- Roff DA, Mousseau TA. 1987.** Quantitative genetics and fitness: lessons from *Drosophila*. *Heredity* **58**: 103–118.
- Santos M, Céspedes W, Belanyà J, Trotta VF, Calboli CF, Fontdevila A, Serra L. 2005.** Temperature-related genetic changes in laboratory populations of *Drosophila subobscura*: evidence against simple climatic-based explanations for latitudinal clines. *The American Naturalist* **165**: 258–273.
- Schmidt PS, Matzkin L, Hipólito M, Eanes WF. 2005.** Geographic variation in diapause incidence, life-history traits and climatic adaptation in *Drosophila melanogaster*. *Evolution* **59**: 1721–1732.
- Sgrò CM, Blows MW. 2004.** The genetic covariance among clinal environments after adaptation to an environmental gradient in *Drosophila serrata*. *Genetics* **167**: 1281–1291.
- Stearns SC. 1992.** *The evolution of life histories*. Oxford: Oxford University Press.
- Tylor BW, Anderson CR, Peckarsky BL. 1998.** Effects of size at metamorphosis on stonely fecundity, longevity and reproductive success. *Oecologia* **114**: 494–502.
- Van Delden W, Kamping A. 1991.** Changes in relative fitness with temperature among second chromosome arrangements in *Drosophila melanogaster*. *Genetics* **127**: 507–514.
- Zwaan B, Bijlsma R, Hoekstra RF. 1995.** Artificial selection for developmental time in *Drosophila melanogaster* in relation to the evolution of aging: direct and correlated responses. *Evolution* **49**: 635–648.