

Developmental time and size-related traits in *Drosophila buzzatii* along an altitudinal gradient from Argentina

PABLO SAMBUCETTI¹, VOLKER LOESCHCKE² and FABIAN M. NORRY¹

¹*Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina*

²*Department of Ecology and Genetics, University of Aarhus, Aarhus, Denmark*

Sambucetti, P., Loeschcke, V. and Norry, F. M. 2006. Developmental time and size-related traits in *Drosophila buzzatii* along an altitudinal gradient from Argentina. — *Hereditas* 143: 77–83. Lund, Sweden. eISSN 1601-5223. Received November 21, 2005. Accepted November 28, 2005

Clinal analysis for fitness-related traits provides a well-known approach to investigate adaptive evolution. Several fitness-related traits (developmental time, thorax length, wing length and wing loading) were measured at two laboratory generations (G7 and G33) of *D. buzzatii* from an altitudinal gradient from northwestern Argentina, where significant thermal differences persist. Developmental time (DT) was positively correlated with altitude of origin of population. Further, DT was negatively correlated with maximal mean temperature at the site of origin of population, and this thermal variable decreases with altitude. Wing loading tended to be larger in highland than in lowland populations, suggesting that flight performance is subject to stronger selection pressure in highland populations. Developmental time showed a significant increase with laboratory generation number. There was no significant correlation between developmental time and body size across populations along the altitudinal cline of DT. This result illustrates that developmental time and body size do not always evolve in the same direction, even though both traits are often positively and genetically correlated in a well-known tradeoff in *Drosophila*.

Fabian M. Norry, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. (1428) Buenos Aires, Argentina. E-mail: fnorry@ege.fcen.uba.ar

Geographic gradients are of special interest for studies on adaptation to contemporaneous terrestrial environments. Evidence for geographic clines in fitness-related traits often comes from variation along latitudinal gradients. In model ectothermal organisms like *Drosophila*, for instance, parallel clines have been identified on different continents for several traits including body size and developmental time, with genetically larger flies at higher latitudes (PARSONS 1980; CAPY et al. 1993; IMASHEVA et al. 1994, 1995; JAMES et al. 1995; AZEVEDO et al. 1998; JAMES and PARTRIDGE 1995; VAN'T LAND et al. 1999; WEEKS et al. 2002). Temperature is typically implied as a selective agent in such latitudinal clines for traits of adaptive importance (REEVE et al. 2000; NORRY et al. 2001).

Developmental time and adult body size are two traits of adaptive importance. On the one hand, faster development can increase fitness in many insects like *Drosophila* either by an increased larval survival under wild conditions or a demographic advantage for early reproduction (ROFF 2000). On the other hand, body size is not a fitness component itself, but increased size often confers an advantage for fecundity and survival in adult flies at non-stressful temperature (PARTRIDGE and FOWLER 1993). On the basis of such fitness associations, developmental time and body size are

connected by a tradeoff because genetically larger individuals develop slower than genetically smaller individuals (ROFF 2000). However, the tradeoff between these traits can disappear at low temperature (NORRY and LOESCHCKE 2002).

Both developmental time and body size are affected by temperature, both decreasing with increasing temperature. Such temperature-induced effects can be the result of phenotypic plasticity (STEARNS 1989). However, both traits also respond to thermal selection in experimental populations (ANDERSON 1966; CAVICCHI et al. 1989). In wild populations of *D. buzzatii*, adaptation to temperature is apparent for developmental time and pre-adult survival but not for size-related traits, suggesting that developmental time is a trait more closely related to thermal adaptation than body size itself (NORRY et al. 2001). However, microevolution of size-related traits such as thorax and wing length in *Drosophila* might be further influenced by functional correlations for flight performance and/or other attributes of adult performance rather than thermal selection (NORRY et al. 1997). For instance, wing loading (usually expressed as the wing/thorax ratio) may have an important adaptive significance in flight performance (STARMER and WOLF 1989; BARKER and KREBS 1995; LOESCHCKE et al. 1999).

In *D. melanogaster*, clinal variation associated with latitude has been demonstrated for several traits along the east coast of Australia, including developmental time (JAMES et al. 1995). However, although developmental time and body size are connected by a tradeoff, the correlation between these traits is often non-significant along latitudinal clines of body size (VAN'T LAND et al. 1999; LOESCHCKE et al. 2000). Therefore, evolution of body size may not be a simple consequence of changes in developmental time.

Although regions at high altitude and latitude share similar conditions of low temperature (LENCIONI 2004), altitudinal gradients have been much less explored than latitudinal gradients in studies of clinal variation in *Drosophila*. However, altitudinal clines can be detected on narrower geographical scales than latitudinal gradients (TATAR et al. 1997; BUBLIY and LOESCHCKE 2005; SØRENSEN et al. 2005).

Here we analyze both developmental time and body-size in the cactophilic *D. buzzatii* along an altitudinal gradient in northwestern Argentina where significant thermal differences persist (Table 1). This species occurs in several phytogeographical areas from lowlands to highlands in Argentina, though its origin is likely to have been in lowlands rather than in highlands in this region. Under the hypothesis that lowland and highland populations have genetically diverged in developmental time and size-related traits, we test for clinal variation between populations across an altitudinal gradient spanning more than 2000 m in height. Previous work failed to detect clinal variation for developmental time and body size along an altitudinal gradient spanning about 1000 m in height for both *D. buzzatii* and *D. simulans* from a Canary Island where *D. buzzatii* was introduced in historical times (BUBLIY and LOESCHCKE 2005). In the present study, we used a wider range of altitude to test for clinal patterns of developmental time and size-related traits in *D. buzzatii* from the region of origin of

this species. Maximal mean temperature for site of origin of population best predicted altitudinal variation in developmental time, with no correlation between developmental time and body size across populations.

MATERIAL AND METHODS

Collection of stocks

Wild flies were collected using banana baits in eight localities of northwestern Argentina in mid-April 2003 (Table 1). Temperature is the main climatic difference between the collection sites. For instance, minimal and maximal mean temperatures in Table 1 represent average daily maximal/minimal temperatures for the coldest/hottest month, using data from the Argentine Meteorological Service web site www.meteofa.mil.ar (temperatures for each particular altitude were obtained by interpolation using records for La Rioja, Salta, Santiago del Estero and La Quiaca). Mass cultures were set up in the field for each original population using about 40 wild caught flies from each locality. One hundred inseminated females of the laboratory G2 generation from each mass culture were transferred to individual vials to set up isofemale lines for each population of origin. *Drosophila koepferae*, a sibling species of *D. buzzatii*, was present at some collection sites and they were identified by examining the male genitalia (VILELA 1983). After four laboratory generations (when all lines were checked for species), 22 isofemale lines of *D. buzzatii* were inter-se crossed within each population, and an experimental stock was established for each population of origin. All stocks were maintained at 25°C on instant *Drosophila* medium (Carolina Biological Supply, Burlington, NC, USA), with 3 bottles per population and 70 to 100 flies per bottle.

Table 1. *Sites of D. buzzatii populations sampled in northwestern Argentina. Estimated minimal and maximal mean temperatures (T_{min} and T_{max} , see Sambucetti et al. 2005 for details) are given for each site studied. Altitude is given in meters above the sea level (m a.s.l.).*

Altitude (m a.s.l.)	Latitude (°)	Longitude (°)	Temperature extremes (°C)	
			T_{min}	T_{max}
202	27.48	64.18	6	34
401	28.52	66.15	4	35
709	33.25	66.25	4	33
739	24.38	65.03	3	33
879	26.08	65.11	3	33
1654	26.06	65.58	1	26
1855	26.27	66.02	0	25
2263	26.35	65.51	-2	24

Measurements and analysis of traits studied.

For each experimental stock or population, first-instar larvae were collected from the laboratory G7 and G33 generations by using small spoons with agar and yeast paste. First-instar larvae were transferred at a density of 100 in 100-ml bottles (2–3 bottles per population) containing 30 ml of culture medium and placed at 25°C. The number of males and females emerging from these cultures was scored every 6 h. Larva to adult developmental time was estimated over the mid-point of each successive interval, pooling all bottles within each stock (as there was no significant variation among bottles, results not shown) in both laboratory generations studied, G7 and G33. For size-related traits, 16 females plus 16 males were randomly chosen from each bottle and subsequently measured for thorax and wing length in each generation studied. Thorax length was measured as the distance between the anterior margin of the thorax and the posterior tip of the scutellum and wing length was measured as the third longitudinal vein length (the distance between points 1 and 5 in LOESCHKE et al. 1999) using a micrometer ocular for both traits. Finally, the ratio of wing length (WL) to thorax length (TL) was calculated as an index of wing loading.

ANOVA was performed for each trait using population, sex and generation as fixed factors. Wing loading was indexed as $(\ln WL - \ln TL)$, hereafter referred to as $\ln (WL/TL)$, as in BARKER and KREBS (1995) to reduce statistical problems arising from ratio measurements (ATCHLEY et al. 1976). Forward step-wise multiple regression analysis was performed using geographical and climatic variables separately to test for clinal variation in the traits studied. All the analyses were performed using the STATISTICA package (STATSOFT 1999).

RESULTS

Developmental time (DT) was substantially faster in laboratory generation G7 than in G33 (Fig. 1, standardized data pooled over population and sex; $F_{1,2225} = 3507.69^{***}$; $***P < 0.005$). Females developed faster than males in both generations studied (Fig. 1, Table 2). DT showed a highly significant interaction between laboratory generation and origin of population (Table 2), and this trait significantly differed among populations in both generations studied (single effect ANOVA: $F_{7,2195} = 7.60^{***}$ for G7; $F_{7,2195} = 136.78^{***}$ for G33; $***P < 0.005$). There was a trend for DT to increase with altitude of origin of population in both generations studied (Fig. 1). Maximal mean temperature in the sites of

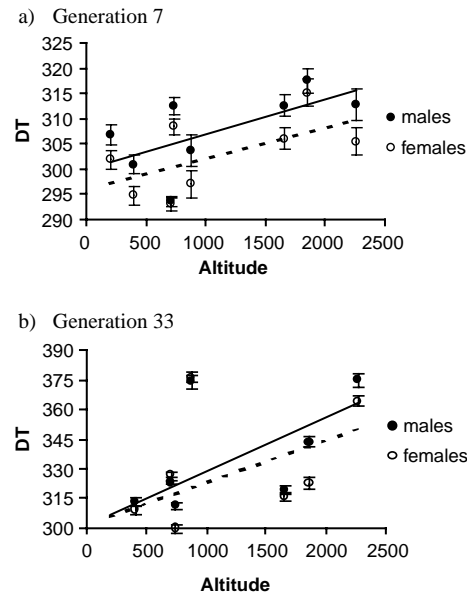


Fig. 1a–b. Developmental time (in hours) for males and females of *D. buzzatii* derived from an altitudinal gradient from northwestern Argentina in laboratory generations (a) G7 and (b) G33. Filled circles and solid lines designate males; while empty circles and dashed lines designate females. Error bars correspond to SE of the mean.

original wild populations significantly predicted DT in G7 (Table 3).

DT showed no significant correlation with size-related traits across populations (Pearson correlation coefficient estimated over population mean values, $r < 0.5$). Significant associations of some traits with either geography or temperature were found in G7 but not in G33 (Table 3), suggesting that this variation disappeared by adaptation to laboratory conditions.

TL was significantly larger in females than in males in both laboratory generations studied (Fig. 2a, Table 2). This trait significantly differed among populations in both generations studied (single effect ANOVA; $F_{7,480} = 3.12^{**}$ for G7; $F_{7,480} = 6.32^{***}$ for G33; $**P < 0.01$; $***P < 0.005$). TL showed no difference between G7 and G33 (data pooled over population and sex; $F_{1,510} = 3.57$). TL showed a significant decrease with altitude of origin of population in G7 males (Fig. 2a, Table 3).

WL was larger in females than in males (Fig. 2b, Table 2). This wing trait showed significant variation among populations in both G7 and G33 (single effects ANOVA: $F_{7,480} = 2.20^*$ for G7; $F_{7,480} = 5.44^{***}$ for G33; $*P < 0.01$; $***P < 0.005$). There was no significant difference in WL between G7 and G33 (standardized data pooled over population and sex, $F_{1,510} = 0.59$, $P > 0.05$). WL significantly increased with latitude but not with altitude of origin of population in both G7 and G33 females (Table 3).

Table 2. ANOVAs performed to test for differences in developmental time (DT), thorax length (TL), wing length (WL) and wing loading [$\ln(WL/TL)$] among populations, sex, laboratory generations and their interactions.

Source of variation	df	DT		TL		WL		$\ln(WL/TL)$	
		MS Effect	F	MS Effect	F	MS Effect	F	MS Effect	F
(1) population	7	60514.58	58.83***	0.44	7.28***	0.76	5.22***	0.008	4.54***
(2) sex	1	14991.04	14.57***	11.34	185.15***	36.99	2.52*	0.001	1.72
(3) generation	1	4483538.51	4359.23***	0.32	5.22*	0.14	0.33	0.029	16.45**
<i>Interactions</i>									
(1) × (2)	7	1148.48	1.12	0.02	0.32	0.18	1.22	0.002	0.52
(1) × (3)	7	60618.89	58.93***	0.13	2.15*	0.35	2.42*	0.001	1.05
(2) × (3)	1	230.41	0.22	0.01	0.15	0.02	0.08	0.001	0.01
(1) × (2) × (3)	7	1014.48	0.98	0.10	1.67	0.28	1.93	0.002	1.30

*P < 0.05; **P < 0.01; ***P < 0.001.

Wing loading [$\ln(WL/TL)$] diverged both between populations and generations, with no significant differences between the sexes (Table 2). Wing loading was larger in G33 than in G7 (Fig. 2c, Table 2). Wing loading increased with altitude of origin of population in G33 but this trend was non-significant in G7 (Fig. 2c, Table 3).

DISCUSSION

Developmental time showed substantial variation among populations of *D. buzzatii* along our altitudinal gradient. Maximal mean temperature at the site of origin of population significantly predicted developmental time. No tradeoff association was apparent

between developmental time and body size across populations along the elevational gradient studied. This result is consistent with previous observations showing that the positive correlation between these traits can disappear when estimated over mean values across populations along latitudinal or altitudinal gradients (VAN'T LAND et al. 1999; LOESCHCKE et al. 2000; NORRY et al. 2001).

The altitudinal variation we found in developmental time presumably reflects genetic variation in developmental rate because all experimental individuals were reared in a common environment. Polymorphic inversions on chromosome 2 are well known to affect both developmental time and body size in *D. buzzatii*, with the 2nd inversion decreasing both developmental

Table 3. Regression coefficients from stepwise multiple regression of traits on geographic and thermal variables, for laboratory-reared *D. buzzatii* from an altitudinal gradient in two laboratory generations: G7 and G33. Traits are developmental time (DT), wing length (WL), thorax length (TL) and wing loading [$\ln(WL/TL)$]. Values are given only for variables that entered in the equation (stepwise method).

Predictor variable and G	DT		TL		WL		$\ln(WL/TL)$	
	males	females	males	females	males	females	males	females
<i>Geography</i>								
G7								
Altitude	0.52*	0.43	-0.89*	-	-0.46	0.28	-	-
Latitude	-0.69*	-0.54	-0.53	0.44	-	0.38*	0.15	-0.42
G33								
Altitude	0.67*	0.51	-	-0.71*	-	-	0.70*	-
Latitude	-	-	-	-0.62	-	0.88*	-	-
<i>Temperature</i>								
G7								
T _{max}	-0.71*	-0.69*	-	-0.10	-	-	-0.81-	-
T _{min}	-	-	0.69	-1.46	-	-	-	-
G33								
T _{max}	-	-	-	0.48	-	-	-0.39	-
T _{min}	-	-	-0.28	-	0.59	-	-	-

*P < 0.05.

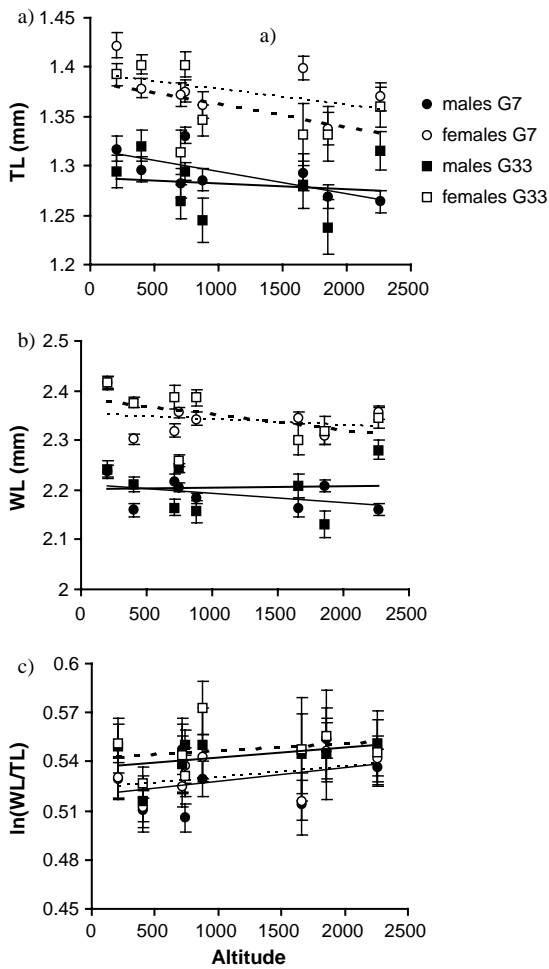


Fig. 2a–c. Size-related traits (a and b) and wing loading (c) for males and females of *D. buzzatii* from an altitudinal gradient from northwestern Argentina in laboratory generations G7 and G33. Thin and thick solid lines designate males in G7 and G33, respectively; while thin and thick dashed lines designate females in generations G7 and G33, respectively. Error bars represents SE of the mean.

time and body size (BETRÁN et al. 1998; NORRY et al. 1995). In addition, this inversion polymorphism clinally varies with altitude of population in the same geographic region included in the present study, with inversion *j* of chromosome 2 increasing in frequency with altitude (HASSON et al. 1995). Thus, the observed trend is consistent with the hypothesis that the genetic basis for altitudinal variation in developmental time can at least partially be based on polymorphic inversions in *D. buzzatii*.

Recently, BUBLIY and LOESCHCKE (2005) found no altitudinal variation for developmental time along an elevational gradient from a Canary island for *D. buzzatii* and *D. simulans*. However, they found that developmental time was longer in the population from the coolest highland locality. Previous work

in another species, *D. melanogaster*, has found that developmental time at 25°C is slower in populations from high as opposed to low latitudes (JAMES and PARTRIDGE 1995; JAMES et al. 1995), which is consistent with evolution of this trait in experimental populations kept at low temperature for many generations (ANDERSON 1966; HUEY et al. 1991; PARTRIDGE et al. 1994, 1995). We found that maximal mean temperature at the site of origin of population consistently predicted variation in developmental time across altitudinal populations (Table 3). This trend disappeared after many laboratory generations, as it was significant in G7 but not in G33 (Table 3).

An altitudinal trend was apparent for thorax length in males in G7 and females in G33, with decreased size in highland populations (Table 3). Wing length increased with latitude (Table 3). Decreased thorax size in highland versus lowland populations of *D. buzzatii* is consistent with previous studies (NORRY et al. 2001). However, the association of size-related traits with climate and/or geography remains unclear in this species. For instance, LOESCHCKE et al. (2000) detected no latitudinal trend for thorax length and various wing measurements in laboratory-reared flies from Australia, although wild-caught flies from the same localities showed a clear-cut latitudinal trend. BUBLIY and LOESCHCKE (2005) found no altitudinal pattern for body size on a Canary island. In the present study we analyzed a larger number of populations and a more extensive altitudinal gradient than in all previous studies, and found a negative relationship between thorax length and altitude of origin of population.

THOMAS and BARKER (1993) and NORRY et al. (2001) showed that wing loading increased with latitude and altitude, respectively, in *D. buzzatii* from Australia and Argentina, and a similar latitudinal trend was also reported by LOESCHCKE et al. (2000). BUBLIY and LOESCHCKE (2005) found no altitudinal variation for this composite trait either in *D. buzzatii* or in *D. simulans* from a Canary island. However, an altitudinal trend was observed for wing loading in the present study (Fig. 2c), but the adaptive significance of such a trend remains unclear because it was significant only in G33 (Table 3). One possible hypothesis to explain an increased wing loading in highland populations is that both feeding and breeding resources available for *D. buzzatii* (rotting tissues of several cactus species) are much less abundant in high elevations than in lowland populations (SAMBUCETTI et al. 2005). Thus, wing loading is expected to increase in highland populations if it confers any advantage for adult flies looking for suitable substrates which are much more patchily distributed in highland than in

lowland populations. However, the results of the present and previous studies discussed above suggest that there is no simple clinal relation between wing loading and altitude of population in *D. buzzatii*. The possible relationship between wing loading and altitude in this species might be further complicated if body size is also influenced by counter gradient selection (CONOVER and SCHULTZ 1995).

Laboratory adaptation is known to increase developmental time in *D. melanogaster* (SGRÒ and PARTRIDGE 2000). Our present results with *D. buzzatii* also showed that development at 25°C was much longer in generation 33 than in generation 7 for all populations, indicating that laboratory adaptation increased developmental time in this species independently of the altitudinal origin of population. In contrast to developmental time, size-related traits were not strongly affected by laboratory adaptation in the present study.

Consistent with previous studies (VAN'T LAND et al. 1999; NORRY et al. 2001; BUBLIY and LOESCHCKE 2005), there was no significant correlation between developmental time and body size across populations along our altitudinal gradient. Developmental time increases with altitude of origin of population. However, developmental time and body size do not typically evolve in the same direction over evolutionary trajectories across populations, even though both traits are positively and genetically correlated in a fundamental tradeoff in *Drosophila*.

Acknowledgements – We are grateful to Stuart Barker for helpful comments on the ms. This research was supported by frame and center grants from the Danish Natural Sciences Research Council to VL, and by grants from the University of Buenos Aires (X139), ANPCyT and CONICET-Argentina to FMN.

REFERENCES

- Anderson, W. W. 1966. Genetic divergence in M. Vetukhiv's experimental populations of *Drosophila melanogaster*. – *Genet. Res.* 7: 255–266.
- Atchley, W. R., Gaskins, C. T. and Anderson, D. 1976. Statistical properties of ratios. I. Empirical results. – *Syst. Zool.* 25: 137–148.
- Azevedo, R. B. R., James, A. C., McCabe, J. et al. 1998. Latitudinal variation of wing: thorax size ratio and wing: aspect ratio in *Drosophila melanogaster*. – *Evolution* 52: 1353–1362.
- Barker, J. F. S. and Krebs, R. A. 1995. Genetic variation and plasticity thorax length and wing length in *Drosophila aldrichi* and *D. buzzatii*. – *J. Evol. Biol.* 8: 689–709.
- Betrán, E., Santos, M. and Ruiz, A. 1998. Antagonistic pleiotropic effect of second-chromosome inversions on body size and early life-history traits in *Drosophila buzzatii*. – *Evolution* 52: 144–154.
- Bubliy, O. A. and Loeschcke, V. 2005. Variation of life-history and morphometrical traits in *Drosophila buzzatii* and *D. 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 variation of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans*. I. Geographic variations. – *Evolution* 25: 517–536.
- Cavicchi, S., Guerra, D., Natali, V. et al. 1989. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. II. Correlation between fitness and body size dimensions. – *J. Evol. Biol.* 2: 235–251.
- Conover, D. O. and Schultz, E. T. 1995. Phenotypic similarity and the evolutionary significance of counter-gradient variation. – *Trends Ecol. Evol.* 10: 248–252.
- Hasson, E., Rodríguez, C., Fanara, J. J. et al. 1995. The evolutionary history of *Drosophila buzzatii*. XXVI. Macrogeographic patterns of inversion polymorphisms in New World populations. – *J. Evol. Biol.* 8: 369–384.
- Huey, R. B., Partridge, L. and Fowler, K. 1991. Thermal sensitivity of *Drosophila melanogaster* responds rapidly to laboratory natural selection. – *Evolution* 45: 751–756.
- Imasheva, A. G., Bubli, O. A. and Lazebny, O. E. 1994. Variation in wing length in Eurasian natural population of *Drosophila melanogaster*. – *Heredity* 72: 508–514.
- Imasheva, A. G., Bubli, O. A., Lazebny, O. E. et al. 1995. Geographic differentiation in wing shape in *Drosophila melanogaster*. – *Genetica* 96: 303–306.
- James, A. C. and Partridge, L. 1995. Thermal evolution of rate of larval development in *Drosophila melanogaster* in laboratory and field populations. – *J. Evol. Biol.* 8: 315–330.
- James, A. C., Azevedo, R. B. R. and Partridge, L. 1995. Cellular basis and developmental timing in a size cline of *Drosophila melanogaster*. – *Genetics* 140: 659–666.
- Lencioni, V. 2004. Survival strategies of freshwater insects in cold environments. – *J. Limnol.* 63 (suppl. 1): 45–55.
- Loeschcke, V., Bundgaard, J. and Barker, J. S. F. 1999. Reactions norms across and genetic parameters at different temperatures for thorax and wing traits in *Drosophila aldrichi* and *D. buzzatii*. – *J. Evol. Biol.* 12: 605–623.
- 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.
- Norry, F. M. and Loeschcke, V. 2002. Temperature induced shifts in association of longevity with body size in *Drosophila melanogaster*. – *Evolution* 56: 299–306.
- Norry, F.M., Vilardi, J.C., Fanara, J.J. et al. 1995. An adaptive chromosomal polymorphism affecting size-related traits, and longevity selection in a natural population of *Drosophila buzzatii*. – *Genetica* 96: 285–291.
- Norry, F. M., Vilardi, J. C. and Hasson, E. 1997. Genetic and phenotypic correlations among size-related traits, and heritability variation between body parts in *Drosophila buzzatii*. – *Genetica* 101: 131–139.
- Norry, F. M., Bubliy, O. A. and Loeschcke, V. 2001. Developmental time, body size and wing loading in *Drosophila buzzatii* from lowland and highland populations in Argentina. – *Hereditas* 135: 35–40.
- Parsons, P. A. 1980. Adaptive strategies in natural populations of *Drosophila*. Ethanol tolerance, desiccation resistance and developmental time in climatically optimal and extreme environments. – *Theor. Appl. Genet.* 57: 257–266.

- Partridge, L. and Fowler, K. 1993. Direct and correlated responses to selection on thorax length in *Drosophila melanogaster*. – *Evolution* 47: 213–226.
- Partridge, L., Barrie, B., Fowler, K. et al. 1994. Thermal evolution of pre-adult life history traits in *Drosophila melanogaster*. – *J. Evol. Biol.* 7: 645–663.
- Partridge, L., Barrie, B., Barton, N. H. et al. 1995. Rapid laboratory evolution of adult life-history traits in *Drosophila melanogaster* in response to temperature. – *Evolution* 49: 538–544.
- Reeve, M.W., Fowler, K. and Partridge, L. 2000. Increased body size confers greater fitness at lower experimental temperature in male *Drosophila melanogaster*. – *J. Evol. Biol.* 13: 836–844.
- Roff, D. A. 2000. Tradeoff between growth and reproduction: an analysis of quantitative genetic evidence. – *J. Evol. Biol.* 13: 434–445.
- Sambucetti, P., Sørensen, J.G., Loeschcke, V. et al. 2005. Variation in senescence and associated traits between sympatric cactophilic sibling species of *Drosophila*. – *Evol. Ecol. Res.* 7: 915–930.
- Sgrò, C. M. and Partridge, L. 2000. Evolutionary responses of life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. – *Am. Nat.* 156: 341–353.
- Sørensen, J. G., Norry, F. M., Scannapieco, A. C. et al. 2005. Altitudinal variation for stress resistance traits and thermal adaptation in adult *Drosophila buzzatii* from the New World. – *J. Evol. Biol.* 18: 829–837.
- Starmer, W. T. and Wolf, L. 1989. Causes of variation in wing loading among *Drosophila* species. – *Biol. J. Linn. Soc.* 28: 373–385.
- Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity. – *BioScience* 39: 436–445.
- StatSoft 1999. STATISTICA for Windows (Computer program manual). – StatSoft Inc., Tulsa.
- Tatar, M., Grey, D. W. and Carey, J. R. 1997. Altitudinal variation for senescence in *Melanophus grasshoppers*. – *Oecologia* 111: 357–364.
- Thomas, R. H. and Barker, J. S. F. 1993. Quantitative genetic analysis of the body size and shape of *Drosophila buzzatii*. – *Theor. Appl. Genet.* 85: 598–608.
- van't Land, J. P., van Putten, P., Zwaan, B. et al. 1999. Latitudinal variation in wild populations of *Drosophila melanogaster*: heritabilities and reactions norm. – *J. Evol. Biol.* 12: 222–232.
- Vilela, C. R. 1983. A revision of the *Drosophila* repleta species (Diptera-Drosophilidae). – *Rev. Bras. Entomol.* 27: 1–114.
- Weeks, A. R., McKechnie, S. W. and Hoffmann, A. A. 2002. Dissecting adaptive clinal variation: markers, inversions and size/stress associations in *Drosophila melanogaster* from a central field population. – *Ecol. Lett.* 5: 756–763.