J. Evol. Biol. 8: 689–709 (1995)

1010–061X/95/060689 21 $ 1.50 + 0.20/0

Genetic variation and plasticity of thorax length and wing length in *Drosophila aldrichi* and *D. buzzatii*

J. S. F. Barker[1]* and R. A. Krebs[2]

[1]*Department of Animal Science, University of New England, Armidale, NSW 2351, Australia*
[2]*Present address: Department of Ecology and Genetics, University of Aarhus, Ny Munkegade, bldg. 540, DK-8000, Aarhus, Denmark*

Key words: Body size; developmental temperature; phenotypic plasticity; reaction norm.

Abstract

Reaction norms across three temperatures of development were measured for thorax length, wing length and wing length/thorax length ratio for ten isofemale lines from each of two populations of *Drosophila aldrichi* and *D. buzzatii*. Means for thorax and wing length in both species were larger at 24 °C than at either 18 °C or 31 °C, with the reduction in size at 18 °C most likely due to a nutritional constraint. Although females were larger than males, the sexes were not different for wing length/thorax length ratio. The plasticity of the traits differed between species and between populations of each species, with genetic variation in plasticity similar for the two species from one locality, but much higher for *D. aldrichi* from the other. Estimates of heritabilities for *D. aldrichi* generally were higher at 18 °C and 24 °C than at 31 °C, but for *D. buzzatii* they were highest at 31 °C, although heritabilities were not significantly different between species at any temperature. Additive genetic variances for *D. aldrichi* showed trends similar to that for heritability, being highest at 18 °C and decreasing as temperature increased. For *D. buzzatii*, however, additive genetic variances were lowest at 24 °C. These results are suggestive that genetic variation for body size characters is increased in more stressful environments. Thorax and wing lengths showed significant genetic correlations that were not different between the species, but the genetic correlations between each of these traits and their ratio were significantly different. For *D. aldrichi*, genetic variation in the wing length/thorax length ratio was due primarily to variation in thorax length, while for *D. buzzatii*, it was due primarily to variation

* Author for correspondence.

in wing length. The wing length/thorax length ratio, which is the inverse of wing loading, decreased linearly as temperature increased, and it is suggested that this ratio may be of greater adaptive significance than either of its components.

Introduction

Most species live in a range of environments, such that different populations may inhabit different environments, and each population may be exposed to different patterns of temporal environmental variation. The responses of populations and of species to such heterogeneous environments may be both phenotypic and genetic, ranging from the rigid development of individuals, where each genotype results in a fixed phenotype which has maximum fitness in one environment (specialization), through all genotypes resulting in a single fixed phenotype that has the highest mean fitness averaged across all environments (canalization), to plastic development, where for a given genotype, the phenotype matches that which is most fit for each environment. Either of these extremes, or some intermediate, may evolve depending on the available genetic variation, i.e. the heritabilities of the trait itself and of its plasticity (Scheiner and Lyman, 1989). Phenotypic plasticity refers to the ability of a genotype to respond differently in different environments, while the array of phenotypes that develop in an array of environments is the reaction norm (Stearns, 1989). The genetics of phenotypic plasticity has been comprehensively reviewed by Scheiner (1993).

From a quantitative genetic viewpoint, reaction norms and phenotypic plasticity are observable phenomena that can be considered primarily through the concept of genotype × environment interactions (Falconer, 1990). In order to understand the evolution of phenotypic plasticity of any character, describing the genetics is an essential first step. However, we need also to determine the character of interest, i.e. what is being or has been selected, and why. The variable expression of a character in different environments may be adaptive, but it is not necessarily so (Gould and Lewontin, 1979).

The cost of flight for an adult *Drosophila* is influenced by the ratio body mass/wing area, or wing loading, and flight capacity also is affected by wing beat frequency. Starmer and Wolf (1989) have shown for a number of *Drosophila* species that wing loading may be indexed by the ratio of linear measurements, thorax length/wing length. Both thorax length and wing length have been commonly used as measures of body size, as both are well known to be highly correlated (both genetically and environmentally) with body mass, either as weight at eclosion or dry weight. Genetic correlations between wing length and thorax length are intermediate to high (Robertson and Reeve, 1952; Thomas and Barker, 1993), but in *D. melanogaster* wing length is known to be subject to genetic variation independent of thorax length (Robertson, 1962). Both traits are highly variable within and between natural populations in several species of *Drosophila* (e.g. Stalker and Carson, 1947, 1948, 1949; McFarquhar and Robertson, 1963; Coyne and Beecham, 1987; Robertson, 1987; Thomas and Barker, 1993).

In natural populations, variation among individuals in body size is likely to be largely environmental, due to variation in larval crowding (Barker and Podger, 1970; Atkinson, 1979; Krebs et al., 1992), food quality (Robertson, 1960; Etges, 1989; Thomas, 1993) and temperature (Parsons, 1961; Tantawy, 1964; Atkinson, 1979; Thomas and Barker, 1993). However, recent studies have shown that the variation in body size within natural populations has a significant genetic component (Coyne and Beecham, 1987; Prout and Barker, 1989; Ruiz et al., 1991).

For *D. melanogaster*, David et al. (1994) have shown that the norms of reaction for thorax length and wing length against temperature during development have the shape of an inverted U, with estimated temperatures at which maximum size is attained of 19.6 °C for thorax length and 15.7 °C for wing length. Thus, as developmental temperature decreases from a maximum consistent with survival to these temperatures, both thorax and wing length increase, but with the change in wing length relatively greater (Misra and Reeve, 1964), due to larger wing size associated with larger wing cells (Robertson, 1959). However, over the full range of temperatures (12 °C–31 °C) at which *D. melanogaster* may complete development, the wing length/thorax length ratio shows a monotonic decrease (David et al., 1994).

The changes in wing and thorax lengths with developmental temperature are reflected in natural populations – with latitudinal variation (Stalker and Carson, 1947; Misra and Reeve, 1964), seasonal variation (Tantawy, 1964; Stalker and Carson, 1949) and altitudinal variation (Stalker and Carson, 1948) all showing relatively longer wings in cooler environments. Stalker and Carson (1947) discussed the possible adaptive significance of this decrease in wind loading for flies from colder habitats, suggesting from the work of Reed et al. (1942) that adults at lower ambient temperatures have lower wing-beat frequency (since confirmed by Unwin and Corbet, 1984). Thus a lower wing loading would benefit adults flying at lower temperatures, while a higher wing loading would not disadvantage adults flying at higher temperatures. Although there may well be genetic variation for wing beat frequency (both between and within populations), for the individual fly it is a physiological response to temperature. Thus it might be argued that wing loading could be a direct target of natural selection (Stalker, 1980; David et al., 1994), and that both genetic and environmental variation affecting thorax and wing lengths may be constrained (Starmer and Wolf, 1989)

Drosophila buzzatii and *D. aldrichi* are both members of the *mulleri* subgroup of the repleta group that breed and feed in rotting cladoles of *Opuntia* spp., and are restricted to this niche. These species colonized Australia some 60 years ago (Barker et al., 1985); *D. buzzatii* from Argentina, and *D. aldrichi* from Texas/northern Mexico, and they are sympatric only in Australia. Since the control of their *Opuntia* hosts in the 1930's by the moth *Cactoblastis cactorum*, the species distributions have been fragmented into numerous isolated or semi-isolated populations (Barker and Mulley, 1976). Thus, as these species have colonized a new habitat and have been sympatric for only some 300–400 generations, they may still be in the process of adapting to new environments and to each other, and diversifying among populations. Genetic differences among populations have been demonstrated for enzyme variation (*D. buzzatii* – Mulley et al., 1979; Sokal et al., 1987; *D. aldrichi* – Krebs

and Barker, 1993, unpubl.), for competitive interactions and body weight (Krebs and Barker, 1993) and for body size of *D. buzzatii* (Robertson, 1987; Thomas and Barker, 1993).

In this paper, we examine genetic variation, heritability and the plasticity of response of thorax length, wing length and their ratio at three temperatures for *D. buzzatii* and *D. aldrichi* from two localities in the region of sympatry.

Materials and methods

Drosophila aldrichi and *D. buzzatii* were collected over banana bait buckets at Oxford Downs (21° 46′ S, 148° 51′ E, *O. tomentosa* along a road side) and Dixalea (23° 56′ S, 150° 17′ E, mixed *O. tomentosa* and *O. stricta* within a wooded area). Within a few hours after collection, flies were sorted to species and sex, and females were placed singly in 75 × 25 mm vials on cactus-yeast-sucrose medium (Starmer and Barker, 1986) to establish isofemale lines.

Effects of temperature on thorax and wing length were studied using ten lines from each locality for each species. Twenty isofemale lines were set up initially, each from more than 20 first generation offspring, which expanded to more than 200 progeny in the second generation. These second generation adults were used to collect eggs over a four hour period on a yeast paste medium (described in Krebs and Barker, 1991) placed in plastic spoons held within half-pint milk bottles. In order to ensure that sufficient larvae were available from ten lines, eggs were collected from twelve, and at the end of the egg collection period, they were rinsed in saline to remove the yeast medium, and transferred to agar plates until hatching. Larvae (<2 hours old) were transferred to vials (six replicates per line per

Table 1. Climatic variables for Oxford Downs and Dixalea – mean daily temperature (TMEAN), mean temperature in summer months (TSUMMER), mean temperature in winter months (TWINTER), mean summer maximum temperature (SUMMAX), mean winter minimum temperature (WINMIN), average daily temperature range (AVTR), annual rainfall (RAIN), seasonality index for temperature (mean January minus mean July temperatures – SEAS-T), and seasonality index for rainfall (rainfall in wettest month minus rainfall in driest month – SEAS-R). All temperatures are in °C, and rainfall in mm. All variables are based on monthly estimates derived by the computer program ESOCLIM, using an interpolation method described by Hutchinson (1989).

Variable	Oxford Downs	Dixalea
TMEAN	22.1	21.6
TSUMMER	26.2	26.2
TWINTER	17.0	15.9
SUMMAX	31.7	31.5
WINMIN	10.5	9.3
AVTR	12.1	12.0
RAIN	812	747
SEAS-T	10.6	11.7
SEAS-R	154.2	108.1

temperature). For each vial, five first-instar larvae were placed on 5g minced and autoclaved *O. tomentosa* that had been seeded 48 hrs previously with six species of bacteria and two of yeast to produce simulated cactus necroses (as in Krebs and Barker, 1991). Vials were placed randomly within two trays that were set side by side, either in an environmental chamber at 31 °C, about 85% relative humidity, or in 18 °C and 24 °C environmental rooms at 70% relative humidity. These temperatures were chosen to cover an ecologically relevant range for populations from the two collection localities (Tab. 1).

The first male and female that emerged from each vial were measured for thorax length (anterior margin of the thorax to the posterior tip of the scutellum, with the dorsal side upwards) and for length of both wings (from the inner angle of the second basal cell to the wing margin of the second longitudinal vein), as described in Robertson and Reeve (1952). A binocular microscope with a digital filar eyepiece (Los Angeles Scientific Instrument Company, Inc.), which logged measurements directly to a microcomputer, was used for all measurements, which were recorded in micrometers. The average length of the two wings was used for all statistical analyses except for the small proportion of flies that had one wing damaged. For these, the measurement for the undamaged wing was used.

Analyses

As in Thomas and Barker (1993), thorax and wing lengths were log transformed for all analyses, and the ratio of wing length to thorax length was analysed as the difference (log wing length – log thorax length) [hereafter referred to as log(W/T)], to reduce statistical problems arising from ratio measures (Atchley et al., 1976). For an overall analysis of variance, lines were treated as random and nested within species × locality. All other effects were fixed: species, locality, temperature and sex. The *F*-tests for the random effect of lines-within-species × locality and all interactions involving this term were constructed against the error mean square, i.e. testing whether the lines differ in marginal means (Fry, 1992). All fixed main effects and their interactions were tested against the appropriate interaction mean square that included lines-within-species × locality, using SAS (SAS, 1989). Because not all vials produced a male and a female, ANOVA using six replicates per line would have been unbalanced. Therefore, one datum for each sex per line (within each species, locality and temperature) was omitted, first eliminating vials where only males or only females were obtained, and if both sexes were obtained from all, one vial was removed randomly. This procedure reduced replicates to five per line. For the few cases where only four replicates of one sex were obtained, the average of those four was used to make up the fifth datum.

As the isofemale lines were initiated from many progeny of each female, and rapidly expanded in size, little genetic variation would have been lost, and the offspring in the first few generations in the laboratory would have an average relatedness of full-sibs. Thus, by treating these isofemale lines as equivalent to full-sib families, an isofemale heritability (H) can be estimated, and from this, the

heritability of individual differences (h^2) derived, together with its standard error (Hoffmann and Parsons, 1988). The intraclass correlations for isofemale lines for each of the three traits at each of the three temperatures and in each species were estimated using LSMLMW, a general purpose mixed model least-squares and maximum likelihood computer program (Harvey, 1982, 1990). Genetic correlations among the three traits also were estimated for each species at each temperature in these analyses. Preliminary analyses had shown no significant differences in heritability for any trait between localities within each species. Therefore in the final model, locality and sex were included as fixed effects, and lines nested in locality as random. Statistical comparisons of heritabilities and genetic correlations were done using the z transformation (Zar, 1984), but substituting the estimated standard errors.

Plasticity for each trait was considered in two ways. In the first, the overall trait mean at each temperature was estimated, and plasticity was measured as the coefficient of variation across temperatures (Schlichting and Levin, 1990). Secondly, plasticity was measured for each pair of temperatures, where it is defined as the difference between the means of each line at the two temperatures (Scheiner and Lyman, 1989). Analysis of plasticity defined in this way allows effects of specific temperature changes to be investigated; effects that may be masked when overall plasticity, as defined above, is considered. Genetic variance in plasticity was evaluated in terms of genotype by environment interactions (line × temperature).

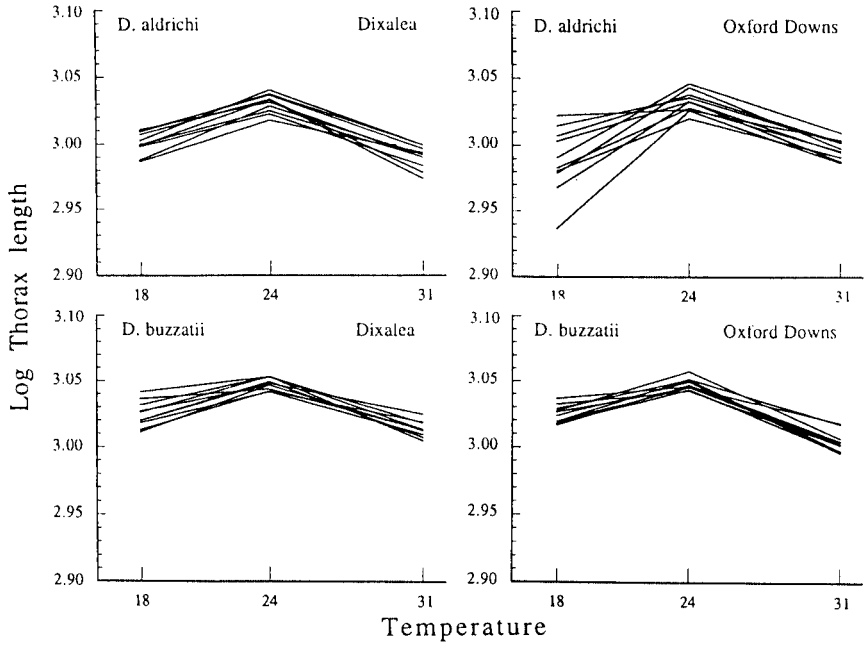

Fig. 1. Reaction norms for log thorax length of isofemale lines of *D. aldrichi* and *D. buzzatii* from Dixalea and Oxford Downs raised at three temperatures. The mean shown for each line at each temperature is the average of five males and five females.

Table 2. Analyses of variance of log thorax length (TL), log wing length (WL), and the log of their ratio (W/T = log wing length − log thorax length) for 10 lines (as a random effect) from each of two localities for *D. aldrichi* and *D. buzzatii* at 18 °C, 24 °C and 31 °C, with one male and one female measured from each of five replicate vials per line.

Source	df[a]	Mean squares × 10^6		
		log(TL)[b]	log(WL)	log(W/T)
Species	1	130044***(1)	95177***	2709
Locality	1	839 (1)	531	36
Spec × Loc	1	192(1)	299	965
Temperature	2	165960***(2)	591632***	325086***
Sex	1	234000***(3)	243339***	92
Line (Spec × Loc)	36(1)	1539***(5)	1287***	667***
Spec × Temp	2	6266***(2)	905	10829***
Spec × Sex	1	4806***(3)	2231**	486
Loc × Temp	2	1572 (2)	2836**	381
Loc × Sex	1	657 (3)	847	12
Temp × Sex	2	1207* (4)	1434**	69
Spec × Loc × Temp	2	5374** (2)	1414	2065**
Spec × Loc × Sex	1	860 (3)	444	68
Temp × Line (Spec × Loc)	72(2)	782***(5)	544***	309***
Sex × Line (Spec × Loc)	36(3)	310 (5)	265	167
Spec × Temp × Sex	2	259 (4)	465	211
Loc × Temp × Sex	2	566 (4)	790*	64
Spec × Loc × Temp × Sex	2	1178* (4)	923*	223
Temp × Sex × Line (Spec × Loc)	72(4)	325 (5)	240	183
Error	960(5)	319	236	159

[a] Numbers in parentheses indicate different denominator mean squares.

[b] Numbers in parentheses indicate the denominator mean square used in *F* tests of each source of variation.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Results

Reaction norms

Temperature significantly affected thorax length, wing length and log(W/T) of *D. aldrichi* and *D. buzzatii* (Tab. 2). Mean thorax length of both species was largest at 24 °C and, except for Oxford Downs *D. aldrichi*, smallest at 31 °C (Fig. 1), while wing length was similar at 18 °C and 24 °C, but much smaller at 31 °C (Fig. 2). Consequently, log(W/T) was essentially linear across the temperature range used in this experiment (Fig. 3).

Drosophila buzzatii had significantly larger thorax and wing lengths than *D. aldrichi*, but the difference in log(W/T) was not significant (Tab. 2). The species × temperature interaction was significant for thorax length and log(W/T), as the difference between the species for thorax length was greater at 18 °C than at 24 °C

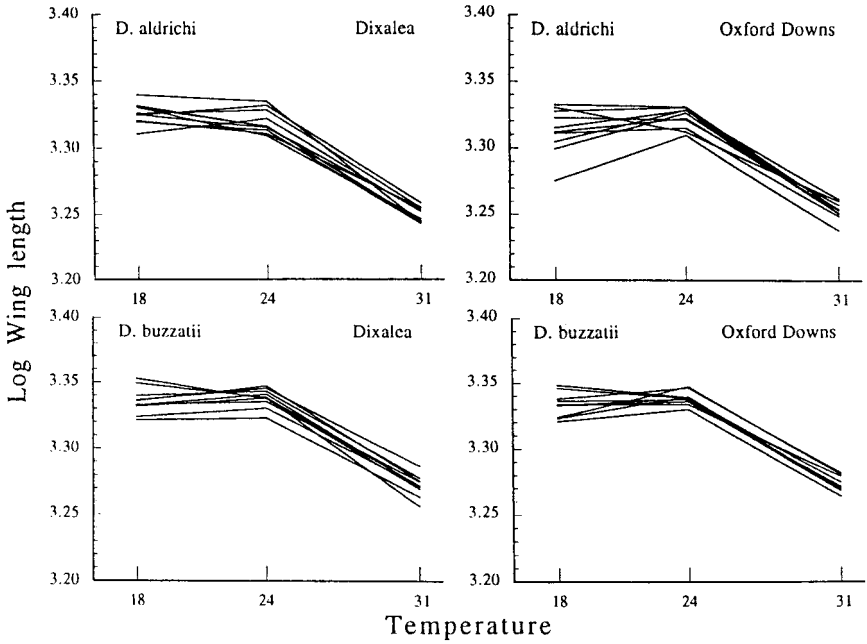

Fig. 2. Reaction norms for log wing length of isofemale lines of *D. aldrichi* and *D. buzzatii* from Dixalea and Oxford Downs raised at three temperatures. The mean shown for each line at each temperature is the average of five males and five females.

or 31 °C, while log(W/T) was higher for *D. aldrichi* at 18 °C, but lower at 31 °C. Locality effects were not significant for any trait.

Thorax and wing lengths of females were larger than those of males, and these sex differences were proportionately larger for *D. buzzatii* as compared with *D. aldrichi* (Tab. 2, significant species × sex interaction). However, there were no significant effects of sex or species × sex for log(W/T). Effects of temperature differed for the two sexes for both thorax and wing length, with larger differences between males and females at 24 °C and 31 °C than at 18 °C.

The line-within-species × locality effect and its interaction with temperature were significant for all three traits. These effects and other significant interactions (particularly species × locality × temperature), are considered in more detail below in terms of genetic variation for these traits and the plasticity over temperatures for each species from each locality.

Plasticity

Differences among populations in amount or pattern of plasticity were indicated by the significant species × locality × temperature interaction for thorax length and

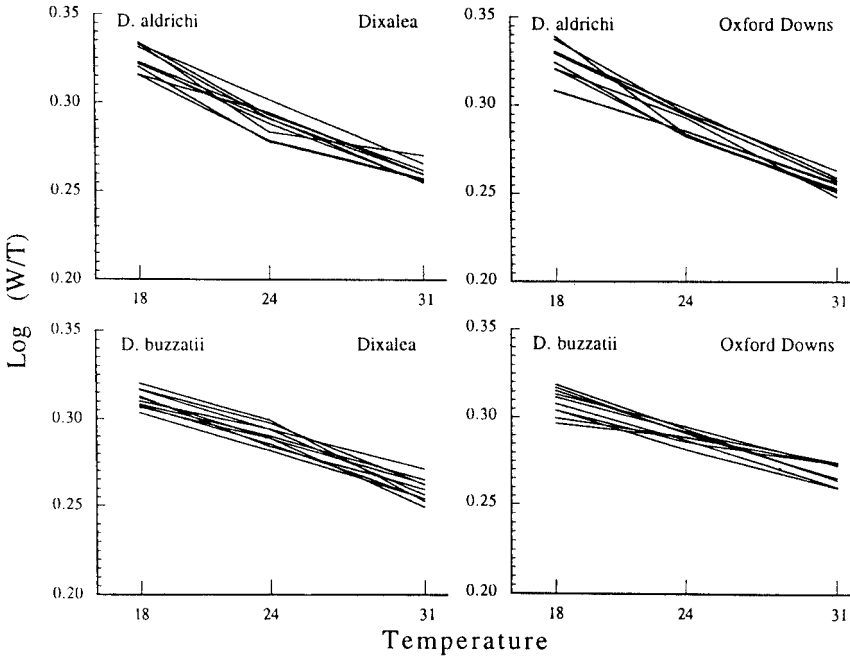

Fig. 3. Reaction norms for log (wing length/thorax length) of isofemale lines of *D. aldrichi* and *D. buzzatii* from Dixalea and Oxford Downs raised at three temperatures. The mean shown for each line at each temperature is the average of five males and five females.

log(W/T) (Tab. 2). Separate ANOVA for each species showed highly significant ($p < 0.001$) locality × temperature interactions for thorax length and wing length in *D. aldrichi*, and for thorax length ($p < 0.01$) and log(W/T) in *D. buzzatii*. The coefficient of variation across temperatures (corrected for bias – Sokal and Rohlf (1981), p. 59) was used as a measure of this plasticity, with higher coefficients representing greater plasticity. With only three temperatures, standard errors of the coefficients were such that none of the following comparisons are significant, and they thus represent only possible trends. For *D. aldrichi*, flies from Oxford Downs had a higher coefficient of variation for thorax length than those from Dixalea (0.847 vs 0.764), but a lower coefficient than those from Dixalea for wing length (1.242 vs 1.389). *Drosophila buzzatii* from Oxford Downs also showed a higher coefficient of variation than those from Dixalea for thorax length (0.795 vs 0.626), and a lower coefficient for wing length (1.196 vs 1.225). For log(W/T), Dixalea *D. buzzatii* showed a higher coefficient than those from Oxford Downs (10.092 vs. 7.727), but for *D. aldrichi*, the coefficient for Dixalea was lower than for Oxford Downs (12.083 vs 12.925).

An alternative measure of plasticity, the differences for the trait means between each pair of temperatures, clarified different patterns of plasticity among the three traits (Tab. 3). For thorax length, the two species were significantly different

Table 3. Mean (±se) for the plasticity (measured as the difference between trait means for each line and each pair of temperatures) of log thorax length, log wing length, and the log of their ratio (W/T), for 10 lines of *D. aldrichi* and *D. buzzatii* from each of two localities, and analyses of variance. Each column gives mean plasticity for a particular pair of temperatures (e.g. 18–24 = mean at 18 °C minus mean at 24 °C), and ANOVA of the 40 line means.

Species	Locality	Thorax length			Wing length			log(W/T)		
		18–24	18–31	24–31	18–24	18–31	24–31	18–24	18–31	24–31
D. aldrichi	Dixalea	−29.7 ± 2.0	11.4 ± 3.7	41.0 ± 3.1	6.0 ± 3.4	76.2 ± 3.8	70.2 ± 3.0	35.6 ± 2.6	64.8 ± 1.9	29.2 ± 2.5
	Oxford Downs	−44.6 ± 7.9	−9.0 ± 6.4	35.6 ± 2.2	−9.5 ± 5.0	60.2 ± 4.1	69.7 ± 2.7	35.0 ± 3.1	69.2 ± 3.3	34.1 ± 1.6
D. buzzatii	Dixalea	−23.6 ± 2.8	10.7 ± 3.6	34.3 ± 2.5	−2.4 ± 2.7	63.8 ± 3.5	66.2 ± 2.3	21.2 ± 1.1	53.1 ± 2.9	31.9 ± 2.5
	Oxford Downs	−24.0 ± 2.6	19.8 ± 3.4	43.7 ± 2.7	−4.8 ± 3.2	60.8 ± 3.2	65.6 ± 1.4	19.2 ± 1.9	41.0 ± 3.4	21.9 ± 1.9
ANOVA		Thorax length			Wing length			log(W/T)		
		18–24	18–31	24–31	18–24	18–31	24–31	18–24	18–31	24–31
Source	Df	Mean squares			Mean squares			Mean squares		
Species	1	1779.2**	1975.4**	5.1	33.2	345.2	164.4	2298.5***	3971.6***	227.3*
Locality	1	582.2	321.1	38.6	793.3*	900.1*	3.4	16.4	147.3	65.3
Spec × Loc	1	530.2	2159.4**	549.6**	431.6	422.0	0.1	5.4	674.6**	559.6**
Error	36	200.0	199.6	70.0	133.8	133.7	58.6	53.0	86.0	46.1

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

($p < 0.01$) for both the 18–24 (i.e. mean at 18 °C minus mean at 24 °C) and 18–31 plasticities, but in different ways. Plasticity was higher for *D. aldrichi* for 18–24, but higher for *D. buzzatii* for 18–31. The significant species × locality interactions ($p < 0.01$) for 18–31 and 24–31 were due to higher plasticity for *D. aldrichi* from Dixalea, but higher for *D. buzzatii* from Oxford Downs. In contrast, plasticity of wing length was not different between the species, but for 18–24 and 18–31, it was higher at Dixalea than at Oxford Downs. Plasticity of log(W/T) was significantly higher for *D. aldrichi* for all three temperature comparisons, while the significant species × locality interactions for 18–31 and 24–31 reflected the differences observed for thorax length.

The significant temperature × line-within-species × locality interaction for all three traits, together with the significant species × temperature interaction for thorax length and log(W/T) (Tab. 2), indicate genetic variation in plasticity, and that this variation may differ between the species. Thus four separate ANOVAs were done – each species from each locality, using LSMLMW and treating temperature and sex as fixed effects, and lines as random. Estimated variance components are given in Table 4, together with the percent contribution of the line × temperature interaction to the total phenotypic variance. As lines were treated as equivalent to full-sib groups, twice this fraction is an estimate of the heritability of plasticity (effectively on a within-environment basis). These heritable components of the plastic variance were similar for the two species from Dixalea (except for log TL), but for Oxford Downs they were substantially higher for *D.*

Table 4. Components of variance for log thorax length (TL), log wing length (WL) and the log of their ratio (W/T) for each locality of each species. Numbers in parentheses are degrees of freedom for the column. V_L = line component, $V_{L\times T}$ = component of line × temperature interaction, V_W = within-line component, V_P = total phenotypic variance (sum of preceding entries). Significance values attached to $V_{L\times T}$ refer to significance of the line × temperature term in the analysis of variance.

Species	Locality	Trait	V_L (9)	$V_{L\times T}$ (18)	V_W (267)	V_P	$V_{L\times T}/V_P$ (%)
D. aldrichi	Dixalea	TL	21.72	3.95	417.08	442.75	0.89
		WL	3.75	29.74**	278.38	311.86	9.54
		W/T	16.31	10.61*	169.89	196.81	5.39
	Oxford Downs	TL	71.14	139.46***	405.46	616.07	22.64
		WL	53.81	53.41***	271.83	379.05	14.09
		W/T	17.74	23.86***	152.98	194.58	12.26
D. buzzatii	Dixalea	TL	6.85	20.44*	248.63	275.91	7.41
		WL	29.82	25.54***	164.93	220.29	11.60
		W/T	8.87	12.61*	135.14	156.61	8.05
	Oxford Downs	TL	1.24	21.56**	204.37	227.16	9.49
		WL	11.73	13.74†	235.15	260.61	5.27
		W/T	4.83	11.92†	186.48	203.23	5.86

† $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 5. Heritabilities (±se) of individual differences (h^2) and the between line component of variance ($V_B = 0.5 \times$ additive genetic variance) for log thorax length (TL), log wing length (WL) and the log of their ratio (W/T) at three temperatures in *D. aldrichi* and *D. buzzatii*.

Trait	Temperature	*D. aldrichi*		*D. buzzatii*	
		h^2	$V_B \times 10^4$	h^2	$V_B \times 10^4$
Log(TL)	18 °C	0.555 ± 0.219*	2.702***	0.211 ± 0.133	0.388**
	24 °C	0.462 ± 0.198*	0.426***	0.061 ± 0.089	0.049
	31 °C	0.331 ± 0.165*	0.416***	0.335 ± 0.167*	0.315***
Log(WL)	18 °C	0.451 ± 0.195*	1.295***	0.340 ± 0.168*	0.590***
	24 °C	0.630 ± 0.236**	0.576***	0.364 ± 0.174*	0.264***
	31 °C	0.278 ± 0.151	0.240**	0.424 ± 0.189*	0.358***
Log(W/T)	18 °C	0.469 ± 0.200*	0.600***	0.139 ± 0.112	0.177*
	24 °C	0.517 ± 0.211*	0.354***	0.263 ± 0.147	0.142**
	31 °C	0.104 ± 0.102	0.074	0.344 ± 0.169*	0.254***

* $p < 0.05$, ** $p < 0.01$.

aldrichi than for *D. buzzatii*. Within species, they were higher for *D. aldrichi* from Oxford Downs, while for *D. buzzatii*, they were generally higher for the Dixalea population.

Quantitative genetic variation

Estimates of heritability for each trait at each temperature in *D. aldrichi* and *D. buzzatii* are given in Table 5, and genetic correlations in Table 6. As the standard errors for the heritabilities and genetic correlations were calculated with no adjustment for the fixed effects, they should be treated as minimum estimates (Harvey, 1990), so that the significance levels shown in Tables 5 and 6 are not conservative. Between line components of variance, together with the significance of the line effect in the ANOVAs, are also shown in Table 5. These show that for all three traits at the three temperatures, additive genetic variances generally were significantly different from zero for both species (except for log(W/T) at 31 °C in *D. aldrichi* and for thorax length at 24 °C in *D. buzzatii*). While there were no significant differences in heritability among temperatures for any trait in either species, nor between the species, heritabilities generally were higher for *D. aldrichi* for all three traits at 18 °C and 24 °C, while for *D. buzzatii*, they were highest at 31 °C. However, differences in heritability estimates among temperatures may reflect differences in genetic variance, environmental variance or both. Additive genetic variances (Tab. 5) for *D. aldrichi* showed trends similar to those for the heritabilities – highest at 18 °C and decreasing as temperature increased. For *D. buzzatii*, on the other hand, additive genetic variances for all three traits were lowest at 24 °C, and generally higher at 18 °C than at 31 °C.

Table 6. Isofemale line genetic correlations (±se) among log thorax length (TL), log wing length (WL) and the log of their ratio (W/T) at each of three temperatures in *D. aldrichi* and *D. buzzatii*.

Species	Temperature	log(TL) and log(WL)	log(TL) and log(W/T)	log(WL) and log(W/T)
D. aldrichi	18 °C	0.908 ± 0.061***	−0.789 ± 0.292**	−0.458 ± 0.306
	24 °C	0.654 ± 0.181***	−0.263 ± 0.328	0.558 ± 0.218*
	31 °C	0.920 ± 0.104***	−0.713 ± 0.663	−0.380 ± 0.480
	overall	0.822 ± 0.096***	−0.630 ± 0.239**	−0.076 ± 0.293
D. buzzatii	18 °C	0.838 ± 0.159***	0.050 ± 0.536	0.588 ± 0.335
	24 °C	0.751 ± 0.369*	0.438 ± 0.808	0.923 ± 0.165***
	31 °C	0.623 ± 0.218**	−0.374 ± 0.389	0.492 ± 0.270
	overall	0.793 ± 0.156***	0.266 ± 0.409	0.799 ± 0.158***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Genetic correlations between thorax length and wing length were significantly different from zero in both species at all three temperatures, but only three other correlations were significant (Tab. 6). For *D. aldrichi*, the thorax length – wing length genetic correlation at 24 °C was significantly less ($p < 0.001$) than those at either 18 °C or 31 °C, while there were no significant differences among temperatures for this correlation in *D. buzzatii*. The only other significant difference among correlations at different temperatures for *D. aldrichi* was for wing length – log(W/T): significantly less ($p < 0.01$) at 18 °C than at 24 °C. For *D. buzzatti*, the genetic correlation between wing length and log(W/T) was significantly higher at 24 °C than either at 18 °C ($p < 0.05$) or at 31 °C ($p < 0.001$).

Given these differences among temperatures, the overall genetic correlations for each species (obtained by including temperature as a fixed effect in the ANOVA model) nevertheless allowed simpler comparison of the species (Tab. 6) The thorax length – wing length genetic correlations for the two species were both significantly greater than zero ($p < 0.001$), but not different between species. However, the genetic correlations between each of these traits and their ratio were significantly different between species. The thorax length – log(W/T) correlation was significantly less than zero for *D. aldrichi* ($p < 0.01$), not different from zero for *D. buzzatii*, and significantly different between species ($p < 0.05$). In contrast, the wing length – log(W/T) correlation was significantly greater than zero for *D. buzzatii* ($p < 0.001$), not different from zero for *D. aldrichi*, and significantly different between species ($p < 0.001$).

Discussion

Reaction norms

Drosophila aldrichi and *D. buzzatii* showed generally similar shaped reaction norms for each of the three traits in response to variation in temperature, with two

general patterns of variation in thorax and wing length observed. Firstly, there were general effects on size that led to similar variation in thorax and wing lengths, and therefore no differences in log(W/T). Large thorax length and wing length differences were found between males and females, as well as several significant interactions that included sex (Tab. 2), but these differences did not lead to variation in log(W/T). A significant temperature × sex interaction for wing length was observed, an effect that was more pronounced for *D. buzzatii* from three other localities (Thomas and Barker, 1993), where wing length of males was affected more by a change in temperature than wing length of females. No interaction terms that included sex and lines-within-species × locality were significant, so that sex differences were similar along all lines of both species and populations. Secondly effects of temperature and lines-within-species × locality, as well as most interactions that included these terms, varied significantly for thorax length and wing length, but differently between the traits. Therefore, the ratio of wing length to thorax length, log(W/T), varied with temperature, and genetic variation was indicated for all three traits in both species. Most importantly, this genetic variation was affected by rearing temperature.

Both thorax length and wing length were longest at the intermediate temperature (24 °C). Previous results indicate that the decreases in thorax length and wing length at 18 °C, as compared with 24 °C, are unlikely to be a direct response to temperature, at least for *D. buzzatii*. Robertson (1987) and Thomas and Barker (1993) raised samples of this species (four different populations in total) at 18 °C and 25 °C under uncrowded conditions on a yeast-cactus-sucrose medium (Starmer and Barker, 1986), and found thorax and wing length were significantly longer at 18 °C. It is most likely that the decrease in body size at 18 °C in the present experiment was due to reduced available nutritional resources. Body size of both species is known to decrease when nutritional resources are reduced, e.g. by increasing larval density on a fixed amount of medium (Krebs and Barker, 1991). In the present experiment, larval density was constant, and with only five larvae per vial, nutritional limitation was not expected. However, the larvae were dependent on the cactophilic yeasts and bacteria seeded onto the autoclaved cactus substrate, and the growth of these microorganisms is substantially reduced at 18 °C, as compared with the higher temperatures (van Uden, 1971). Also, given the long developmental period at 18 °C (28 days to first adult emergence), the cactus substrate had partially dried out by the time pupae were first observed. Such desiccation of the substrate would further slow or stop microorganism growth, so that there could well have been effective resource limitation. While this effect of an interaction between temperature and nutritional limitation was fortuitous, it is very relevent to natural populations, as Thomas (1993) has shown that body size of *D. buzzatii* in nature is at least 25% smaller than predicted for optimal nutrition.

In *D. mulleri*, the thorax length of flies grown at 18 °C was only slightly longer than that for flies grown at 22 °C (Starmer and Wolf, 1989). Both this finding, and the lower body weights of *D. viracochi* grown at 15 °C as compared with 25 °C (Hunter, 1965) also may have been due to nutritional effects, or they may simply reflect the norms of reaction for these species. Nevertheless, these postulated

nutrition-temperature interactions warrant further study, particularly as Gebhardt and Stearns (1993) found similar or slightly lower body weights of *D. melanogaster* raised at 22 °C than at 25 °C for flies raised on their high and medium yeast concentrations, but significantly higher weights at 22 °C than at 25 °C for the low yeast concentration. We suggest that a negative relationship between body weight and temperature would be strongest without nutritional limitation, i.e. high yeast concentration. Yet their results show a small positive correlation at the high yeast level over the temperature range from 22 °C to 28 °C. There is no obvious reason for the results of Gebhardt and Stearns (1993), except that they might be specific to the genetic material used, viz. six genetically homogeneous lines.

As well as temperature-nutrition interactions affecting body size, temperature effects *per se* need to be considered in more detail. In a study that covered the full range of temperatures (12 °C–31 °C) at which *D. melanogaster* may complete growth, David et al. (1994) estimated temperatures at which maximum size was attained as 19.6 °C for thorax length and 15.7 °C for wing length. The temperature reaction norms for body size traits in *D. buzzatii* may well be displaced to maxima at higher temperatures, as this species is adapted to higher temperatures than are *D. melanogaster* and other temperate species (Parsons, 1981; Eid-Dib et al., 1993). Further, the temperature at which body size is maximized might be expected to be higher for *D. aldrichi*, as this species is adapted to higher temperatures than *D. buzzatii* (Krebs and Barker, 1993). The greater decrease in thorax length from 25 °C to 18 °C for *D. aldrichi* than for *D. buzzatii* is consistent with this hypothesis.

Plasticity

Amounts and patterns of plasticity, which differed between the species, were most obvious when analyzed in terms of pair-wise temperature comparisons. For thorax length, *D. aldrichi* showed greater plasticity than *D. buzzatii* for the 18 °C–24 °C comparison, but *D. buzzatii* showed greater plasticity for the 18 °C–31 °C comparison. These differences may relate to the presumed stress effects of temperature on these species – *D. aldrichi* being affected more (greater stress) at 18 °C, and *D. buzzatii* affected more at 31 °C. de Jong (1990) has developed a quantitative genetic approach to the analysis of reaction norms, defining the heritability of plasticity as the additive genetic variance in slopes of linear reaction norms divided by the total variation in slopes. As we have data for only 10 isofemale lines from each population of each species, heritabilities of plasticity defined in this way have not been estimated. However, genotype × environment interaction is closely related to the product of the additive variance in slopes of linear reaction norms and the variance in the environmental variable. Thus indicative estimates of the heritable component of the plastic variance have been obtained from the genotype × environment interaction (line × temperature) component of variance (Tab. 4). Averaging over traits, the heritable component of plasticity is somewhat larger for *D. aldrichi*. Within species, there are no differences between populations for *D.*

buzzatii, but for *D. aldrichi* the interaction component is much greater for all three traits for Oxford Downs than for Dixalea.

Quantitative genetic variation

Heritabilities (Tab. 5) were not significantly different between the two species for any of the three traits, and they were generally consistent with previous estimates of heritabilities of these traits for other *Drosophila* species (Roff and Mousseau, 1987), and for *D. buzzatii* (Robertson, 1987; Thomas and Barker, 1993). Neither were the heritabilities significantly different between temperatures, but there were trends that suggested important differences between the two species. For all three traits, estimates of heritability were higher for *D. aldrichi* at 18 °C and 24 °C than at 31 °C, but for *D. buzzatii*, they were highest at 31 °C. These trends are negatively related to results of competition and fitness component studies, where *D. buzzatii* was generally superior at 25 °C (Krebs and Barker, 1991), but *D. aldrichi* at 31 °C (Krebs and Barker, 1993). Hoffmann and Parsons (1991, pp. 117–131) review changes in heritability with increasing levels of stress, and discuss possible reasons why heritability might increase or decrease. Our results are suggestive that the heritability of body size in these two species increases at temperatures that are less favorable (i.e. higher stress). Any such changes in heritability with increasing stress may be due to changes in the genetic variance, the environmental variance, or both. If, as we infer, 18 °C is the most stressful temperature for *D. aldrichi*, then additive genetic variance for these body size traits clearly increases in more stressful environments. However, the relationship is less clear for *D. buzzatii*, where additive genetic variances were higher at both 18 °C and 31 °C than at 24 °C. While 31 °C is more stressful for this species than 24°C, we have no evidence that 18 °C also is a stress environment.

Large genetic correlations between thorax and wing lengths (Tab. 6) were consistent across the three temperatures, but genetic correlations for either thorax length or wing length with log(W/T) were not consistently significant. This suggests that genetic mechanisms controlling the allometric relationship between wing and thorax lengths vary with the environment. The correlations between wing length and log(W/T) for both species were higher at 24 °C than at either 18 °C or 31 °C, but no other differences or trends were apparent between the species. However, the overall genetic correlations (i.e. temperature included as a fixed effect), showed a major contrast between the species for the correlations between log(W/T) and each of the components – thorax length and wing length (Tab. 6). Even though the correlation between thorax and wing lengths was high (around 0.8) in both species, the thorax length – log(W/T) correlation was significantly negative in *D. aldrichi*, but zero in *D. buzzatii*, while the wing length – log(W/T) correlation was zero in *D. aldrichi*, but significant and positive in *D. buzzatii*. Given the high positive correlations between thorax and wing lengths, one might expect in both species strong negative correlations between thorax length and log(W/T) and strong positive correlations between wing length and log(W/T), as was found for *D. buzzatii* by

Thomas and Barker (1993). However, when both the primary traits and their ratio are log transformed as here, the relative magnitudes of the genetic correlation between the ratio and its numerator (WL), as compared with that between the ratio and its denominator (TL) depend on the genetic correlation between WL and TL and the square root of the ratio additive genetic variance for TL/additive genetic variance for WL (derived from Turner and Young, 1969, pp. 203–207). Thus the correlations observed here imply that for *D. aldrichi*, genetic variation in log(W/T) is due primarily to variation in thorax length, while for *D. buzzatii*, it is due primarily to variation in wing length.

With regard to the different genetic correlations between thorax length and log(W/T) for *D. buzzatii* in Thomas and Barker (1993) and here – strong negative and not significantly different from zero respectively – the populations studied by Thomas and Barker (1993) were from localities some 480 to 1200 km south of Oxford Downs and Dixalea, and the genetic basis of these body size traits may vary along a latitudinal cline, just as does body size itself.

The possibility of selection

The cost of transport for an adult *Drosophila* is influenced by the ratio of wing area to body mass (wing loading), which we measured as its inverse, viz. log(W/T). As this trait varies with the environment of development, wing loading is likely to be subject to genetic or evolutionary changes that may produce both intra- and interspecific variation within and among closely related species (Starmer and Wolf, 1989). However, developmental pathways for thorax length and wing length are different. Thorax length is determined primarily by larval growth, but wing length is determined in part by the size at pupation, and in part by the temperature during the pupal stage (Starmer and Wolf, 1989). Both traits are subject to general effects of growth, again noting large genetic correlations between thorax and wing lengths at any given temperature (Tab. 6).

Much of the recent interest in phenotypic plasticity and reaction norms has been in the context of the evolution of life histories (Roff, 1992; Stearns, 1992). Most life history traits (e.g. fertility, fecundity, survival) are recognized as components of fitness in population genetics models, but life history theory seems generally to include body size also as a life history trait. However, body size *per se* is not a component of fitness, although it will commonly show significant correlations (both phenotypic and genetic) with one or more components of fitness. In a number of *Drosophila* species, adult body size is positively correlated with various adult fitness components – fecundity (Robertson, 1957; Partridge, 1988), mating success (Markow, 1985; Partridge et al., 1987a, b; Pitnick, 1991; Santos et al., 1988), and dispersal ability (Roff, 1977, but see Gu and Barker, 1995), while male wing length is subject to sexual selection, at least in the laboratory (Wilkinson, 1987). To the extent that these correlations are genetic, larger body size implies higher adult fitness at any temperature (Cavicchi et al., 1989). Obviously as average body size remains stable in natural populations, there must be trade-offs with other fitness components, but the nature of these is unknown (David et al., 1994).

Comparing the shape of the reaction norms of the three traits in both species (Figs. 1, 2 and 3), only that for log(W/T) was linear, a result also found by Thomas (1993) for *D. buzzatii*. This linear relationship was found despite possible nutritional effects that reduced body size at 18 °C. A model for the evolution of reaction norm shape under selection (Gavrilets and Scheiner, 1993) suggests that the reaction norm with the highest mean fitness in a temporally varying environment is linear. The one necessary assumption of this model is that the environment of selection is correlated with the environment of development. For selection to act on the reaction norm of log(W/T), wing length must be adjusted developmentally for thorax length. This adjustment is possible for *Drosophila*, because developmentally, wing length is determined after thorax length and only a short time before emergence of adults, five days after the onset of pupation at 25 °C. Therefore, the average temperature experienced by the emerging adults is likely to be similar to that encountered by pupae. However, is selection likely to be acting to adjust the relative body size (thorax length) and wing size (wing length), in terms of the ratio (W/T)? The variation in wing size due to temperature during the pupal stage is purely a function of wing cell area (Robertson, 1959). On the other hand, wing cell number, determined earlier during the larval stage, may be more closely correlated with thorax size than is wing size itself. It is conceivable then that variation in wing cell area due to temperature is a physiological response unrelated to the importance of wing loading. Clearly what is needed is evidence showing whether genetic variation in wing cell area response to temperature is independent of thorax length. Further, we emphasise that the linear reaction norm we observed for log(W/T) is not due to temperature alone, but is a compound effect of temperature and restricted nutrition. Robertson and Reeve (1952) noted that in *D. melanogaster*, W/T decreases when flies are reared at higher temperatures, but that it increases when body size is reduced by a restriction of food supply.

As noted earlier, wing beat frequency increases with ambient temperature. However, David et al. (1994) report preliminary experiments on *D. melanogaster* showing that wing beat frequency of flies tested at 21 °C increased with increased temperature of development. Consideration of the adaptive significance of wing loading (or its inverse as the ratio W/T) will require data on the genetic relationships between wing loading and wing beat frequency. Nevertheless, it is clear that body size *per se*, as either thorax length or wing length alone, is not sufficient to characterize adaptive differences between populations.

Acknowledgements

We thank Annette Edmonds and Don Fredline for technical assistance, Jennifer Krebs for assistance with the figures, and Freddy Christiansen, Volker Loeschcke and Forbes Robertson for critical review of the manuscript. The work was supported by a grant from the Australian Research Council to J. S. F. Barker.

References

Atchley, W. R., C. T. Gaskins and D. Anderson. 1976. Statistical properties of ratios. I. Empirical results. Syst. Zool. 25: 137–148.

Atkinson, W. D. 1979. A field investigation of larval competition in domestic *Drosophila*. J. Anim. Ecol. 48: 91–102.

Barker, J. S. F. and J. C. Mulley. 1976. Isozyme variation in natural populations of *Drosophila buzzatii*. Evolution 30: 213–233.

Barker, J. S. F. and R. N. Podger. 1970. Interspecific competition between *Drosophila melanogaster* and *Drosophila simulans*: effects of larval density on viability, developmental period and adult body weight. Ecology 51: 170–189.

Barker, J. S. F., F. de M. Sene, P. D. East and M. A. Q. R. Pereira. 1985. Allozyme and chromosomal polymorphism of *Drosophila buzzatii* in Brazil and Argentina. Genetica 67: 161–170.

Cavicchi, S., D. Guerra, V. Natali, C. Pezzoli and G. Giorgi. 1989. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. II. Correlation between fitness and body dimensions. J. Evol. Biol. 2: 235–251.

Coyne, J. A. and E. Beecham. 1987. Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. Genetics 117: 727–737.

David, J. R., B. Moreteau, J. P. Gauthier, G. Pétavy, A. Stockel and A. G. Imasheva. 1994. Reaction norms of size characters in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis. Génét. Sél. Evol. 26: 229–251.

de Jong, G. 1990. Quantitative genetics of reaction norms. J. Evol. Biol. 3: 447–468.

Eid-Dib, C., B. Moreteau, G. Vannier, Y. Carton and J. R. David. 1993. Tolerance to a progressive desiccation stress in *Drosophila melanogaster* and *D. buzzatii* populations from a desert oasis. Drosophila Inform. Serv. 72: 115–116.

Etges, W. J. 1989. Evolution of developmental homeostasis in *Drosophila mojavensis*. Evol. Ecol. 3: 189–201.

Falconer, D. S. 1990. Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance. Genet. Res., Camb. 56: 57–70.

Fry, J. D. 1992. The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. Evolution 46: 540–550.

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

Gebhardt, M. D. and S. C. Stearns. 1993. Phenotypic plasticity for life history traits in *Drosophila melanogaster*. I. Effects on phenotypic and environmental correlations. J. Evol. Biol. 6: 1–16.

Gould, S. J. and R. C. Lewontin. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc. R. Soc. Lond. B 205: 581–598.

Gu, H. and J. S. F. Barker. 1995. Genetic and phenotypic variation for flight ability in the cactophilic *Drosophila* species, *D. aldrichi* and *D. buzzatii*. Ent. Exp. appl. (in press).

Harvey, W. R. 1982. Mixed model capabilities of LSML76. J. Anim. Sci. 54: 1279–1285.

Harvey, W. R. 1990. User's Guide for LSMLMW and MIXMDL PC-2 Version. Copyright W. R. Harvey.

Hoffmann, A. A. and P. A. Parsons. 1988. The analysis of quantitative variation in natural populations with isofemale strains. Génét. Sél. Evol. 20: 87–98.

Hoffmann, A. A. and P. A. Parsons. 1991. Evolutionary Genetics and Environmental Stress. Oxford University Press, Oxford.

Hunter, A. S. 1965. Effects of temperature on *Drosophila* II. Respiration of *D. pseudoobscura* and *D. viracochi* grown at different temperatures. Comp. Biochem. Physiol. 16: 7–12.

Hutchinson, M. F. 1989. A new objective method for spatial interpolation of meteorological variables from irregular networks applied to the estimation of monthly mean solar radiation, temperature, precipitation and windrun. CSIRO Division of Water Resources, Tech. Memo. 89/5: 95–104.

Krebs, R. A. and J. S. F. Barker. 1991. Coexistence of ecologically similar colonising species: Intra- and interspecific competition in *Drosophila aldrichi* and *D. buzzatii*. Aust. J. Zool. 39: 579–593.

Krebs, R. A. and J. S. F. Barker. 1993. Coexistence of ecologically similar colonising species. II. Population differentiation in *Drosophila aldrichi* and *D. buzzatii* for competitive effects and responses at different temperatures and allozyme variation in *D. aldrichi*. J. Evol. Biol. 6: 281–298.

Krebs, R. A., J. S. F. Barker and T. P. Armstrong. 1992. Coexistence of ecologically similar colonising species III. *Drosophila aldrichi* and *D. buzzatii*: larval performance on, and adult preference for, three *Opuntia* cactus species. Oecologia 92: 362–372.

McFarquhar, A. M. and F. W. Robertson. 1963. The lack of evidence for co-adaptation in crosses between geographical races of *Drosophila subobscura*. Coll. Genet. Res., Camb. 4: 104–131.

Markow, T. A. 1985. A comparative investigation of the mating system of *Drosophila hydei*. Anim. Behav. 33: 775–781.

Misra, R. K. and E. C. R. Reeve. 1964. Clines in body dimension in populations of *Drosophila subobscura*. Genet. Res., Camb. 5: 240–256.

Mulley, J. C., J. W. James and J. S. F. Barker. 1979. Allozyme genotype-environment relationships in natural populations of *Drosophila buzzatii*. Biochem. Genet. 17: 105–126.

Parsons, P. A. 1961. Fly size, emergence time and sternopleural chaeta number in *Drosophila*. Heredity 16: 455–473.

Parsons, P. A. 1981. Evolutionary ecology of Australian *Drosophila*: A species analysis. Evol. Biol. 14: 297–350.

Partridge, L. 1988. Lifetime reproductive success in *Drosophila*, pp. 11–23. *In* T. H. Clutton-Brock (ed.), Reproductive Success. University of Chicago Press, Chicago.

Partridge, L., A. Ewing and A. Chandler. 1987a. Male size and mating success in *Drosophila melanogaster*: the roles of male and female behaviour. Anim. Behav. 35: 555–562.

Partridge, L., A. Hoffmann and J. S. Jones. 1987b. Male size and mating success in *Drosophila melanogaster* and *D. pseudoobscura* under field conditions. Anim. Behav. 35: 468–476.

Pitnick, S. 1991. Male size influences mate fecundity and remating interval in *Drosophila melanogaster*. Anim. Behav. 41: 735–745.

Prout, T. and J. S. F. Barker. 1989. Ecological aspects of the heritability of body size in *Drosophila buzzatii*. Genetics 123: 803–813.

Reed, S. C., C. M. Williams and L. E. Chadwick. 1942. Frequency of wing-beat as a character for separating species races and geographic varieties of Drosophila. Genetics 27: 349–361.

Robertson, F. W. 1957. Studies in quantitative inheritance XI. Genetic and environmental correlation between body size and egg production in *Drosophila melanogaster*. J. Genet. 55: 428–443.

Robertson, F. W. 1959. Studies in quantitative inheritance. XII. Cell size and number in relation to genetic and environmental variation of body size in Drosophila. Genetics 44: 869–896.

Robertson, F. W. 1960. The ecological genetics of growth in *Drosophila* 1. Body size and developmental time on different diets. Genet. Res., Camb. 1: 288–304.

Robertson, F. W. 1962. Changing the relative size of the body parts of *Drosophila* by selection. Genet. Res., Camb. 3: 169–180.

Robertson, F. W. 1987. Variation of body size within and between wild populations of *Drosophila buzzatii*. Genetica 72: 111–125.

Robertson, F. W. and E. C. R. Reeve. 1952. Studies in quantitative inheritance I. The effects of selection of wing and thorax length in *Drosophila melanogaster*. J. Genet. 50: 414–448.

Roff, D. 1977. Dispersal in Dipterans: Its costs and consequences. J. Anim. Ecol. 46: 443–456.

Roff, D. A. 1992. The Evolution of Life Histories; Theory and Analysis. Chapman and Hall, New York.

Roff, D. A. and T. A. Mousseau. 1987. Quantitative genetics and fitness: lessons from *Drosophila*. Heredity 58: 103–118.

Ruiz, A., M. Santos, A. Barbadilla, J. E. Quezada-Diaz, E. Hasson and A. Fontdevila. 1991. Genetic variance for body size in a natural population of *Drosophila buzzatii*. Genetics 128: 739–750.

Santos, M., A. Ruiz, A. Barbadilla, J. E. Quezada-Diaz, E. Hasson and A. Fontdevila. 1988. The evolutionary history of *Drosophila buzzatii*. XIV. Larger flies mate more often in nature. Heredity 61: 255–262.

SAS 1989. SAS/STAT User's Guide. SAS Institute, Cary, N.C.

Scheiner, S. M. 1993. Genetics and evolution of phenotypic plasticity. Annu. Rev. Ecol. Syst. 24: 35–68.

Scheiner, S. M. and R. F. Lyman. 1989. The genetics of phenotypic plasticity I. Heritability. J. Evol. Biol. 2: 95–107.

Schlichting, C. D. and D. A. Levin. 1990. Phenotypic plasticity in *Phlox*. III. Variation among natural populations of *P. drummondii*. J. Evol. Biol. 3: 411–428.

Sokal, R. R., N. L. Oden and J. S. F. Barker. 1987. Spatial structure in *Drosophila buzzatii* populations: simple and directional spatial autocorrelation. Amer. Nat. 129: 122–142.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. W. H. Freeman and Co., San Francisco.

Stalker, H. D. 1980. Chromosome studies in wild populations of *Drosophila melanogaster*. II. Relationship of inversion frequencies to latitude, season, wing-loading and flight activity. Genetics 95: 211–223.

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

Stalker, H. D. and H. L. Carson. 1948. An altitudinal transect of *Drosophila robusta* Sturtevant. Evolution 2: 295–305.

Stalker, H. D. and H. L. Carson. 1949. Seasonal variation in the morphology of *Drosophila robusta* Sturtevant. Evolution 3: 330–343.

Starmer, W. T. and J. S. F. Barker. 1986. Ecological genetics of the *Adh-1* locus of *Drosophila buzzatii*. Biol. J. Linn. Soc. 28: 373–385.

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

Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity. BioScience 39: 436–445.

Stearns, S. C. 1992. The Evolution of Life Histories. Oxford University Press, Oxford.

Tantawy, A. O. 1964. Studies on natural populations of *Drosophila*. III. Morphological and genetic differences of wing length in *Drosophila melanogaster* and *D. simulans* in relation to season. Evolution 18: 560–570.

Thomas, R. H. 1993. Ecology of body size in *Drosophila buzzatii*: Untangling the effects of temperature and nutrition. Ecol. Entomol. 18: 84–90.

Thomas, R. H. and J. S. F. Barker. 1993. Quantitative genetic analysis of the body size and shape of *Drosophila buzzatii*. Theor. Appl. Genet. 85: 598–608.

Turner, H. N. and S. S. Y. Young. 1969. Quantitative Genetics in Sheep Breeding. Macmillan of Australia, South Melbourne.

Unwin, D. M. and S. A. Corbet. 1984. Wingbeat frequency, temperature and body size in bees and flies. Physiol. Entomol. 9: 115–121.

van Uden, N. 1971. Kinetics and energetics of yeast growth, pp. 75–118. *In* A. H. Rose and J. H Harrison (eds.), The Yeasts Vol. 2. Physiology and Biochemistry of Yeasts. Academic Press, London.

Wilkinson, G. S. 1987. Equilibrium analysis of sexual selection in *Drosophila melanogaster*. Evolution 41: 11–21.

Zar, J. H. 1984. Biostatistical Analysis. 2nd edn. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Received 6 July 1994;
accepted 5 January 1995.
Corresponding Editor: S. Scheiner