Theor Appl Genet (1993) 85:598–608

Quantitative genetic analysis of the body size and shape of *Drosophila buzzatii*

Richard H. Thomas* and J. S. F. Barker

Department of Animal Science, University of New England, Armidale, NSW 2351, Australia

Received August 15, 1992; Accepted September 19, 1992
Communicated by G. Wenzel

Summary. Body size in *Drosophila* is known to be closely related to a number of traits with important life history consequences, such as fecundity, dispersal ability and mating success. We examine the quantitative genetic basis of body size in three populations of the cactophilic species *Drosophila buzzatii*, which inhabit climatically different areas of Australia. Flies were reared individually to eliminate any common environmental component in a full-sib design with families split between two temperatures (18° and 25 °C). The means of several size measures differ significantly among populations while the genetic correlations among these traits generally do not differ, either among populations from different natural environments or between the different laboratory temperatures. This stability of correlation structure is necessary if laboratory estimates of genetic correlations are to have any connection with the expression of genetic variation in the field. The amount of variance due to genotype-by-environment interactions (family × temperature of development) varied among populations, apparently in parallel with the magnitudes of seasonal and diurnal variation in temperature experienced by the different populations. A coastal population, inhabiting a relatively thermally benign environment, showed no interaction, while two inland populations, inhabiting thermally more extreme areas, showed interaction. This interaction term is a measure of the amount of genetic variation in the degree of phenotypic plasticity of body size in response to temperature of development. Thus the inland flies vary in their ability to attain a given body size at a particular temperature while the coastal flies do not. This phenotypic plasticity is shown to be due primarily to differences among genotypes in the amount of response to the change in temperature. A possible selective basis for the maintenance of genetic variation for the levels of phenotypic plasticity is proposed.

Key words: Phenotypic plasticity – Body size – *Drosophila buzzatii* – Development temperature – Genotype × environment interactions

Introduction

Laboratory studies of genetic and phenotypic variation in size and shape in *Drosophila* have a long history (e.g., Robertson and Reeve 1952; Reeve and Robertson 1953), while recent work has provided the first estimates of the heritability of body size in nature (Coyne and Beecham 1987; Prout and Barker 1989; Ruiz et al. 1991). Further, a number of traits with important life history consequences are known to be related to body size; for example, both fecundity (Robertson 1957; Atkinson 1979) and dispersal ability (Roff 1977) in *D. melanogaster* are positively correlated with female body size. Wilkinson (1987) has demonstrated sexual selection for increased male wing length in a laboratory population of *D. melanogaster* recently derived from the wild. This sexual selection was shown to be countered by viability selection. Partridge et al. (1987b) have observed an advantage in mating success for larger males in field populations of *D. melanogaster* and *D. pseudoobscura*. Laboratory studies detected a genetic component in this effect in *D. melanogaster* and indicated its behavioral bases (Partridge et al. 1987a). Santos et al. (1988) have observed that larger *Drosophila buzzatii* mate more often in wild populations, suggesting that they are more likely to encounter suitable mates than smaller flies.

* *Present address:* Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK
Correspondence to: J. S. F. Barker

For many species, different populations inhabit environments with very different patterns of spatial and temporal variation, and these populations may respond, both genetically and phenotypically, in a variety of ways to these differing demands from their environments. At its simplest, we can think of a continuum of response ranging from development along a rigidly stereotyped path regardless of external environment to a broadly plastic phenotypic response to the vagaries of the external environment. Knowledge of the genetic bases of these responses is necessary for understanding evolution in variable environments (Gupta and Lewontin 1982; Via and Lande 1985). Genetic variation may allow a species to respond to variation in the environment, with changes in trait-means increasing adaptation. On the other hand, phenotypic plasticity might allow a species to cope with environmental variation. These are not to be seen as alternatives, but may often be complementary (Bradshaw 1965). Phenotypic plasticity is related to the concept of the norm of reaction of a genotype – i.e., the array of phenotypes that the genotype would develop over an array of environments. Stearns (1989) gives a detailed discussion of the reaction-norm concept. For just two environments, the norm of reaction can be viewed as a plot of the mean phenotypes in the two environments, while the amount of plasticity is the difference between the mean phenotypes in the two environments. Phenotypic plasticity itself may show genetic variation (Scheiner and Goodnight 1984; Via 1984; Scheiner and Lyman 1989), and the phenotypic plasticity of a trait may evolve independently of the trait mean (Schlichting and Levin 1990). A theory for the quantitative genetics of reaction norms has been introduced by de Jong (1990a), while Gabriel and Lynch (1992) and Gomulkiewicz and Kirkpatrick (1992) develop different theoretical approaches to the study of the evolution of reaction norms.

Phenotypic plasticity and reaction norms also relate to the concept of genotype × environment interactions, which has been extensively utilized in applications of quantitative genetics to plant and animal breeding (Pani and Lasley 1972; Falconer 1989). There, however, the primary interest has not been in the magnitude of change from one environment to another (amount of plasticity), nor in the genetic variation for plasticity itself, but on the rankings of strains or of individuals in different environments, on differences in heritability of the trait in different environments, and on the specification of optimum selection and breeding programs.

Genotype-by-environment interactions will be present when a change in environment does not have the same effect on all genotypes, but such interaction may take several forms (Haldane 1946; Dickerson 1962; Bowman 1972). Thus a specific change from one environment to another may have a greater effect on some genotypes than on others, i.e., differences in the amount of response or differences between environments in the intra-environment variation. Alternatively, the rank order of genotypes may be different in the two environments, i.e., differences in the amount or direction of response. In this case, selection in each of the two environments would favor different genotypes. These two forms are not mutually exclusive, and both may contribute to an observed interaction.

There are a number of methods which may be used to evaluate genotype-by-environment interactions; viz., (1) analysis of variance to test the significance of the genotype-by-environment term, (2) estimation of variance components from this ANOVA, and specification of the interaction component as a percentage of the phenotypic variance (this value is related to the heritability of phenotypic plasticity as defined by Scheiner and Lyman 1989), (3) estimation of the correlation between family means for a particular trait in the two environments, and (4) graphical presentation of the norms of reaction, that is, the mean phenotype for each family in the two environments. The first two methods indicate total interaction effects due to differences in the amount of response and in the direction of response. The correlation between family means (method 3) will be unity if the change in environment has the same effect on all genotypes (i.e., no interaction) and will still be unity if there are strictly proportional or multiplicative genetic and environmental effects (i.e., the interaction involves only differences in the amount of response). Robertson (1959) considered a correlation of 0.8 or less to be of "biological significance", which we interpret as indicating substantial differences in the direction of response. A formal genetic treatment of the relationship between the genotype × environment interaction variance approach and that of the genetic correlation between trait values in two environments is given by de Jong (1990b). Method (4) provides for visual evaluation.

Here we examine the quantitative genetics of body size and shape in three populations of *D. buzzatii* inhabiting different climatic areas, and will address three main questions. First, we determine whether populations of *D. buzzatii* have differentiated genetically for body size and shape measures, and if so, to what environmental factors the responses are related. Next, we consider plasticity at different temperatures for these traits in the three populations and their genetic bases. Finally, we compare genetic correlations among traits across the populations and laboratory temperatures. Clark (1987) has suggested that, for phenotypically plastic traits, genetic correlations will not be stable across environments. If this were generally true, it would be very difficult indeed to make evolutionary predictions for populations (Scheiner et al. 1991).

Our experimental animal, *D. buzzatii*, is a member of the *mulleri* subgroup of the repleta group, and is completely dependent on rotting cactus tissue and fruits for

feeding and breeding substrates. Since the control of its *Opuntia* hosts in Australia in the 1930s by the moth *Cactoblastis cactorum*, the range of *D. buzzatii* has been fragmented into numerous isolated or semi-isolated populations (Barker and Mulley 1976). The discrete nature of its substrate, its fragmented range, and its known history of introduction make *D. buzzatii* an excellent subject for genetical and ecological study.

Materials and methods

Sites

The *D. buzzatii* populations analyzed were from three sites where *O. stricta* was the only host plant species, viz.: (1) Hemmant (27°27′S, 153°3′E), located about 1 km from the coast on the outskirts of Brisbane, Queensland, (2) O'Hara (32°26′S, 150°39′E), a small number of plants located inland in an open paddock in the Hunter Valley near Denman, New South Wales, some 650 km from Hemmant, and (3) Trinkey (31°22′S, 149°27′E), hundreds of plants located near Tambar Springs, N.S.W., in a sheltered forest-paddock edge about 150 km NNW of O'Hara. Summaries of climate data (Table 1) show that the study sites differ for a range of variables, but the patterns of differences are not consistent for all. For example, Trinkey is most extreme for the Seasonality index (temperature), while Hemmant is most extreme for the Seasonality index (rainfall).

Collection and maintenance of populations

Wild flies were collected over banana baits and maintained in the laboratory in 75 × 25 mm vials on the cactus-yeast-agar medium described in Starmer and Barker (1986). In order to maintain a large effective population size, virgin progeny from each of a large number of wild females (100–200) were randomly mated to the progeny of another wild female and cultured under uncrowded conditions. In the following F_2 and F_3 generations progeny from these pair matings were isolated as virgins and crossed at random to progeny from other lines. Thus inbreeding was minimized.

Characters studied

Measurements were made on anaesthetized flies as described in Robertson and Reeve (1952). A binocular microscope with a digital filar eyepiece (Los Angeles Scientific Instrument Co., Inc.) which logged measurements directly to a microcomputer was used for all measurements. The characters measured were:

(1) Thorax length (TL): measured from the anterior margin of the thorax to the posterior tip of the scutellum, with the fly oriented dorsal side upward.
(2) Proximal wing length (WP): measured from the inner angle of the 2nd basal cell to the intersection of the anterior cross vein with the third longitudinal vein.
(3) Distal wing length (WD): measured from the intersection of the anterior cross vein with the third longitudinal vein to the wing margin at the distal end of the third longitudinal vein.
(4) Wing length (WL): the sum of WP and WD.
(5) Wing length to thorax length ratio (W/T): WL/TL.

Experimental design

Genetic variation for the above traits was examined by means of a full-sib design with families (12 individuals) split between two temperatures, 18 and 25 °C, both maintained with a 12:12 light:dark cycle and 70% relative humidity. Because it was not possible to determine the sex of sibs when the treatments were initiated, the data are unbalanced with respect to sex. Some of the inherent problems with a full-sib design were minimized, as family members were moved as eggs or early first instar larvae to individual small vials (45 × 15 mm) eliminating any component due to a common environment (Robertson 1987). Maternal effects on adult body size may be disregarded (Robertson 1987). Work on *D. melanogaster* suggests that dominance deviations for body size are of minor importance in outbred populations (Robertson and Reeve 1955). With somewhat less justification, the epistatic terms are generally assumed to be negligible (Falconer 1989). Thus the sib correlations in this study are assumed to reflect primarily the additive genetic component.

The Hemmant and O'Hara experiments were run simultaneously but for logistical reasons were broken into two halves, one using the F_2 generation and the other the F_3 generation. The Trinkey experiment was run at a later date and used the F_2 generation only.

Analysis

Data were analyzed for each population under two multiway mixed-model unbalanced designs. The analyses were carried out using LSMLMW, a general purpose mixed model least-squares and maximum likelihood computer program (Harvey 1982, 1988). Variance components were estimated using Henderson's method 3 in this program.

The first model was

$$Y_{ijkm} = \mu_{jkm} + F_i + T_j + S_k + TS_{jk} + \varepsilon_{ijkm} \quad (1)$$

where μ_{jkm} = population mean, F_i = random effect due to the i^{th} family, T_j = fixed effect of the j^{th} temperature, S_k = fixed effect due to the k^{th} sex, TS_{jk} = effect of the interaction between the j^{th} temperature and the k^{th} sex, and ε = error term. The second

Table 1. Summaries of climatic data derived from weather stations located near the study sites

	AV-T	SEAS-T	SEAS-R	SD-WT	SUMMAX	WINMIN	AVTRY	RAIN
O'Hara	17.50	13.130	7.078	4.750	29.53	4.89	13.30	629
Trinkey	18.30	16.973	2.660	6.129	34.33	3.12	16.11	535
Hemmant	20.53	10.075	21.363	3.636	29.33	10.22	10.04	1,043

AV-T, average annual temperature; SEAS-T, seasonality index (temperature) = mean January av. daily temp. − mean July av. daily temp.; SEAS-R, seasonality index (rainfall) = mean January rain − mean July rain; SD-WT, standard deviation of weekly mean temp. (over the 52 weeks of the year); SUMMAX, av. of weekly av. maximum temps. for the 13 summer weeks; WINMIN, av. of weekly av. minimum temps. for the 13 winter weeks; AVTRY, av. temp. range (daily max. − daily min.) for each week averaged over the 52 weeks; RAIN, total/year (mm)

model was used to examine genotype-by-environment interactions across temperatures, but separately for each sex. This model was

$$Y_{ijm} = \mu_{jm} + F_i + T_j + FT_{ij} + \varepsilon_{ijm} \quad (2)$$

where the notation is as above. Model (2) also was used to estimate heritabilities and genetic and phenotypic correlations, but with measurements scaled to remove effects of sexual dimorphism in size, allowing the pooling of data of both sexes (Falconer and King 1953).

Because of the dependence of variances on means, all data were log-transformed. The transformation resulted in the independence of means and variances for all characters, as verified by regressing family means on their variances. Transforming the ratio of wing length to thorax length results in the difference log (WL)−log (TL) [hereafter referred to as log (W/T)], thereby eliminating the statistical problems arising from ratio measures (Atchley et al. 1976). Standard errors for heritabilities and genetic correlations were calculated with no adjustment for fixed effects and, therefore, they should be considered as minimum estimates (Harvey 1988). Phenotypic correlations were calculated as given in Harvey (1988). Statistical comparisons of correlations were done using the z transformation (Zar 1984), but substituting the estimated standard errors.

Results

Population comparisons

Analyses of variance for both Hemmant and O'Hara that incorporated the two halves of the experiment as a treatment showed no significant differences ($P > 0.1$) for any character. For the remaining analyses, the halves were pooled within sites. Table 2 gives the overall means for thorax and wing length at each site for each temperature and sex and indicates means which were not significant different. Hemmant flies were uniformly smaller than those from O'Hara and Trinkey. At 18 °C O'Hara and Trinkey flies did not differ, but at 25 °C Trinkey flies were very slightly, but significantly and consistently, smaller than O'Hara flies. Since the Trinkey experiment was run at a later time than the Hemmant and O'Hara experiments, these differences might be due to uncontrolled variation in the temperature regime or food composition, though our records show no evidence of temperature or humidity variations in the temperature-controlled rooms and every effort was made to ensure consistent batches of food. Also, as mentioned above, the two halves of the Hemmant and O'Hara experiments showed no effect due to time of conduct of the experiment. Wing length to thorax length ratios, as a measure of shape (Table 3), showed that Hemmant flies were not only smaller than flies from the other populations but also had relatively smaller (shorter) wings in relation to body size. O'Hara and Trinkey flies were not significantly different for this measure. Thus, assuming wing area is proportional to wing length (Starmer and Wolf 1989), and given that thorax length is highly correlated with body mass (Robertson and Reeve 1952; Starmer and Wolf 1989), Hemmant flies had higher wing loading than O'Hara and Trinkey flies.

Results from analyses of variance for each population using model (1) above are shown in Table 4. The main effects, family, temperature and sex, were all very highly significant ($P < 0.001$), except for the effect of sex on the ratio of wing to thorax length [log (W/T)], which was nonsignificant ($P > 0.05$) in all populations. Interaction effects of temperature-by-sex were nonsignificant ($P > 0.05$) for thorax length in all populations, indicating that size in both sexes was similarly affected by different temperatures of development. Wing length on the other hand, showed very highly significant temperature-by-sex interaction effects in all populations, confirming Robertson's (1987) observation that male wing length was relatively more affected by different temperatures of development. As expected from these results, the wing-length to thorax-

Table 2. Comparisons of mean thorax length and mean wing length between populations reared individually in small vials at either 18° or 25 °C. Measurements are in micrometers on a log scale. Vertical bars connect means which are not significantly different using the Student-Newman-Keuls multiple range procedure with $P < 0.05$

Population	Females				Males			
	18 °C		25 °C		18 °C		25 °C	
	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N
Thorax length								
Hemmant	3.0850 ± 0.0008	139	3.0709 ± 0.0007	146	3.0515 ± 0.0007	132	3.0368 ± 0.0007	137
O'Hara	3.0912 ± 0.0005	298	3.0752 ± 0.0004	339	3.0573 ± 0.0005	311	3.0410 ± 0.0005	309
Trinkey	3.0917 ± 0.0007	135	3.0734 ± 0.0006	167	3.0574 ± 0.0008	147	3.0379 ± 0.0007	177
Wing length								
Hemmant	3.3931 ± 0.0010	139	3.3624 ± 0.0007	146	3.3622 ± 0.0007	132	3.3266 ± 0.0007	137
O'Hara	3.4038 ± 0.0005	298	3.3716 ± 0.0004	339	3.3718 ± 0.0005	311	3.3357 ± 0.0004	309
Trinkey	3.4037 ± 0.0007	135	3.3694 ± 0.0006	167	3.3718 ± 0.0008	147	3.3327 ± 0.0006	177

Table 3. Average (log wing–log thorax length) at different temperatures of development. Vertical bars connect means which are not significantly different using the Student-Newman-Keuls multiple range procedure with $P<0.05$. Measurements are in micrometers on a log scale

Site	Females		Males	
	18 °C	25 °C	18 °C	25 °C
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Hemmant	0.3082 ± 0.0009	0.2914 ± 0.0007	0.3107 ± 0.0008	0.2898 ± 0.0006
O'Hara	0.3126 ± 0.0004	0.2964 ± 0.0004	0.3145 ± 0.0005	0.2947 ± 0.0004
Trinkey	0.3120 ± 0.0007	0.2960 ± 0.0006	0.3144 ± 0.0008	0.2948 ± 0.0007

Table 4. Analyses of variance under model (1) for each population. See text for further information

Measure	Source	Hemmant		O'Hara		Trinkey	
		df	MS	*df*	MS	*df*	MS
Log (TL)	Family	57	209.347***	122	228.697***	72	227.888***
	Temperature	1	26,728.195***	1	82,463.691***	1	52,793.644***
	Sex	1	138,537.377***	1	332,090.893***	1	159,214.072***
	Temperature × sex	1	2.200	1	3.518	1	5.652
	Remainder	493	57.763	1,131	54.147	550	58.065
Log (WL)	Family	57	200.059***	122	231.110***	72	210.014***
	Temperature	1	139,447.143***	1	63,050.652***	1	192,543.598***
	Sex	1	136,592.267***	1	27,275.898***	1	162,898.474***
	Temperature × sex	1	740.044***	1	1,240.259***	1	690.901***
	Remainder	493	64.387	1,131	45.793	550	44.781
Log (WP)	Family	57	725.156***	122	768.951***	72	729.562***
	Temperature	1	268,016.806***	1	670,619.512***	1	347,437.470***
	Sex	1	150,511.061***	1	316,744.875***	1	176,448.597***
	Temperature × sex	1	302.440	1	1,193.354***	1	404.804
	Remainder	493	122.463	1,131	113.908	550	128.169
Log (WD)	Family	57	329.263***	122	355.572***	72	307.653***
	Temperature	1	92,001.426***	1	248,355.959***	1	133,855.170***
	Sex	1	130,038.630***	1	332,419.573***	1	156,607.347***
	Temperature × sex	1	1,055.507***	1	1,237.516***	1	861.132***
	Remainder	493	102.528	1,131	57.289	550	62.063
Log (W/T)	Family	57	288.060***	122	243.367***	72	245.680***
	Temperature	1	44,074.227***	1	99,459.451***	1	43,693.217***
	Sex	1	6.876	1	17.581	1	21.072
	Temperature × sex	1	661.542***	1	1,111.664***	1	571.573***
	Remainder	493	53.876	1,131	42.031	550	52.777

*** Significant at the 0.1% probability level

length ratio showed very significant ($P<0.001$) temperature-by-sex interaction effects in all populations. The significant temperature-by-sex interaction for wing length was due primarily to distal wing length (interaction significant in all populations) rather than proximal wing length (interaction significant only for O'Hara).

Quantitative genetic variation

Estimates of heritabilities, and genetic and phenotypic correlations, calculated on data scaled to remove the effect of sexual dimorphism in size (Table 5), were generally similar for all three populations, although heritabilities for wing length and distal wing length were lower for Hemmant than for O'Hara or Trinkey. Robertson (1987) reported much lower heritabilities for both thorax and wing length for Hemmant than for O'Hara. The calculation of standard errors for his heritability estimates (his Table 9) permits comparison with our results. Since Robertson (1987) estimated heritabilities at 25 °C, we calculated heritabilities and genetic and phenotypic correlations separately for flies reared at 18 °C and 25 °C. Herita-

Table 5. Heritabilities and genetic and phenotypic correlations for five traits in each of the three populations. Standard errors should be regarded as minimum estimates. Heritabilities are on the diagonal, genetic correlations are above the diagonal, and phenotypic correlations are below it

	Log (TL)	Log (WL)	Log (WP)	Log (WD)	Log (W/T)
Log (TL)					
Hemmant	0.424±0.098	0.147±0.189	−0.255±0.162	0.390±0.166	−0.692±0.153
O'Hara	0.392±0.063	0.337±0.111	0.332±0.105	0.119±0.119	−0.603±0.114
Trinkey	0.478±0.095	0.472±0.122	0.295±0.141	0.364±0.136	−0.505±0.163
Log (WL)					
Hemmant	0.478	0.324±0.088	0.550±0.128	0.711±0.093	0.615±0.119
O'Hara	0.527	0.394±0.063	0.481±0.089	0.738±0.053	0.548±0.087
Trinkey	0.471	0.584±0.101	0.575±0.100	0.805±0.052	0.522±0.114
Log (WP)					
Hemmant	0.280	0.545	0.666±0.114	−0.197±0.177	0.606±0.113
O'Hara	0.420	0.633	0.634±0.075	−0.237±0.108	0.112±0.111
Trinkey	0.337	0.601	0.639±0.103	−0.023±0.149	0.277±0.142
Log (WD)					
Hemmant	0.397	0.866	0.055	0.326±0.088	0.209±0.174
O'Hara	0.380	0.838	0.108	0.524±0.070	0.519±0.086
Trinkey	0.351	0.824	0.043	0.608±0.102	0.436±0.125
Log (W/T)					
Hemmant	−0.478	0.543	0.277	0.486	0.604±0.110
O'Hara	−0.526	0.446	0.191	0.439	0.550±0.072
Trinkey	−0.576	0.450	0.216	0.408	0.524±0.097

bilities were generally higher at 18 °C than at 25 °C (Table 6), significantly so for all traits at O'Hara, while the differences were significant only for log (WD) at Hemmant (higher at 25 °C) and for log (WP) at Trinkey (higher at 18 °C). Heritabilities for thorax length and wing length were not significantly different in comparing Robertson's "test 1" and our results at 25 °C. His "test 2" heritability for Hemmant thorax length was marginally significantly different from ours at the 5% level. However, since the standard errors for both populations were very likely under-estimates, not much weight can be placed on this result.

Plasticity

Plasticity differences among populations: differences among populations in amounts or patterns of plasticity were tested using a modified model (1) ANOVA including data for all three populations, and with family nested within population. The population × temperature interaction term was highly significant ($P < 0.001$) for log (TL), log (WL) and log (WD), and significant ($P < 0.05$) for log (WP) but not for log (W/T). For all four traits showing significant interaction, the difference between means at 18 °C and 25 °C (amount of plasticity) within popula-

Table 6. Heritabilities with standard errors and variance components for between-family means at 18 and 25 °C for each of three populations

Population	18 °C		25 °C	
	$h^2 \pm SE$	Variance component	$h^2 \pm SE$	Variance component
Hemmant				
Log (TL)	0.493±0.130	18.460	0.390±0.122	14.151
Log (WL)	0.419±0.126	20.225	0.435±0.124	13.443
Log (WP)	0.781±0.134	75.401	0.655±0.132	58.666
Log (WD)	0.261±0.116	20.103	0.690±0.132	34.604
Log (W/T)	0.570±0.132	26.838	0.704±0.132	22.382
O'Hara				
Log (TL)	0.666±0.089	26.029	0.447±0.082	14.491
Log (WL)	0.794±0.090	27.908	0.642±0.087	18.652
Log (WP)	0.863±0.089	82.022	0.748±0.088	62.435
Log (WD)	0.899±0.088	46.011	0.693±0.088	24.865
Log (W/T)	0.879±0.089	28.711	0.587±0.086	17.163
Trinkey				
Log (TL)	0.529±0.129	21.533	0.566±0.117	21.299
Log (WL)	0.648±0.131	24.226	0.549±0.117	15.220
Log (WP)	0.929±0.125	92.474	0.663±0.119	65.755
Log (WD)	0.656±0.131	35.311	0.616±0.118	23.742
Log (W/T)	0.738±0.130	30.102	0.605±0.118	21.302

tions was greatest for Trinkey, intermediate for O'Hara, and least for Hemmant.

Genotype by environment interactions: analysis of variance using model (2) above gave significant genotype (family)-by-environment (temperature) interactions for all characters in the O'Hara population. The only significant interaction terms found in the other populations were for log (WP). As in the analysis under model (1), main effects accounted for most of the variance. More informative, however, were the estimated variance components under model (2), given in Table 7 for the females of each population. The results for males were similar. Both O'Hara and Trinkey showed equivalent and fairly substantial contributions of interaction variance to the total phenotypic variance. At Hemmant, the phenotypic variance was higher than in the other two populations, and the interaction component was positive, but small, only for log (WP).

Table 8 presents correlations between family means in the two environments, calculated as:

$$r_m = \frac{cov_{m(XY)}}{\sqrt{var_{m(X)}\, var_{m(Y)}}} \quad (3)$$

where $cov_{m(XY)}$ is the covariance of the family means of the character measured in the two environments, X and Y, and $var_{m(X)}$ and $var_{m(Y)}$ are the variances of the family means in temperatures X and Y respectively. For these calculations, the scaled data were used, and measurements of both sexes were pooled. Values of the correlation coefficient ranged from about 0.35 to 0.65 and in all cases were highly significantly different from both zero and one ($P<0.01$). As noted by Via (1984), this estimate of a genetic correlation across environments is likely to be an underestimate due to sampling error in the numerator and contamination of the denominator by within-family error variance. However, Spearman rank correlations between family means in the two temperatures were highly significantly less than one ($P<0.005$) for log (TL), log (WL) and log (W/T), and plots of family means in the two environments show many cases of change in rank between environments. Thus for these three traits in all three populations, this analysis indicates that genotypes (families) vary in their direction of response to different temperatures.

This interaction, as indicated by the correlation between family means, is not reflected in the variance component analysis for the Hemmant population (Table 7). Thus the substantial contributions of interaction variance to the total phenotypic variance for O'Hara and Trinkey are interpreted to mean that these interactions are due primarily to differences among families within each of these populations in the amount of response to the change in temperature. This is supported by comparisons of the variance components for among-family means at 18 °C and 25 °C (Table 6), where this variance was sub-

Table 7. Components of variance for body measurements. Numbers in parentheses are degrees of freedom for the column. V_I = variance component (additive and nonadditive) of genotype × environment interaction; V_F = family component; V_e = within-family variance component; V_P = total phenotypic variance (sum of preceding entries). Negative values of V_I are set to zero for calculation of V_I/V_P

	V_I (45)	V_F (56)	V_e (182)	V_P	V_I/V_P (%)
Hemmant females					
Log (TL)	−4.589	16.777	63.948	80.725	0
Log (WL)	−5.931	17.458	81.923	99.381	0
Log (WP)	4.131	61.402	134.928	200.461	2.1
Log (WD)	−15.083	23.452	142.447	165.899	0
Log (W/T)	−2.081	26.241	65.179	91.420	0
O'Hara females					
	(106)	(121)	(408)		
Log (TL)	9.767	11.126	53.949	74.842	13.1
Log (WL)	11.478	12.522	48.579	72.579	15.8
Log (WP)	16.006	58.030	112.801	86.837	8.6
Log (WD)	12.416	23.590	57.920	93.926	13.2
Log (W/T)	7.439	18.752	40.969	67.160	11.1
Trinkey females					
	(54)	(70)	(176)		
Log (TL)	−1.865	14.745	48.339	63.084	0
Log (WL)	3.325	19.637	35.935	58.897	5.6
Log (WP)	18.978	85.390	91.987	96.355	9.7
Log (WD)	8.821	24.921	57.907	91.649	9.6
Log (W/T)	3.692	20.533	41.392	65.617	5.6

Table 8. Genetic correlations, r_m, between family means measured in different environments (temperatures). Data are scaled to remove sex differences in size. Numbers of families, N, in these analyses are less than those in the ANOVAs because only families with two or more offspring surviving at each temperature were included. All values are highly significantly different from both zero and one ($P<0.01$). See text for further explanation

Population	Measure	r_m	N
Hemmant	Log (TL)	0.5044	50
	Log (WL)	0.3578	50
	Log (W/T)	0.6450	50
O'Hara	Log (TL)	0.4816	113
	Log (WL)	0.4174	113
	Log (W/T)	0.5515	113
Trinkey	Log (TL)	0.5165	56
	Log (WL)	0.6576	56
	Log (W/T)	0.5931	56

stantially higher at 18 °C for all five traits for O'Hara, for all but log (TL) for Trinkey, but only for log (WL) for Hemmant. Further, the magnitude of the increase in variance at 18 °C (expressed as a ratio – 18 °C/25 °C) closely corresponds to the percentage V_I/V_P (Table 7), with average values of 1.626 (five traits for O'Hara), 1.475 [four traits, excluding log (TL) for Trinkey], and 1.285 for log (WP) for Hemmant. Thus there is significant genetic variation in the phenotypic plasticity of response to different temperatures of development for the two inland populations, but not for the coastal population.

The most obvious difference between the coastal site Hemmant, which showed no genotype × environment interaction for the traits measured, and the two inland sites, O'Hara and Trinkey, which did show an interaction effect, is climate. Summary climatic data (Table 1) show that Hemmant is more thermally benign that the other sites, both diurnally and seasonally.

Comparisons of genetic correlations among populations and temperatures

In all populations, phenotypic correlations and the corresponding genetic correlations were generally of the same sign and often of similar magnitude, except for the correlations of log (WP) and log (WD) where, in all populations, the genetic correlation was negative and the phenotypic positive.

Comparing populations, the genetic correlations were very similar, with only three cases of significant differences. For log (TL) and log (WP), the correlation in the Hemmant population was negative and significantly different ($P < 0.01$) from the positive correlations for O'Hara and Trinkey. The correlation for log (WP) and log (W/T) also differs between Hemmant and O'Hara ($P < 0.001$) and between Hemmant and Trinkey ($P < 0.05$). For log (WL) and log (WD), the correlation in the Trinkey population was significantly greater ($P < 0.05$) than in each of the other populations.

The genetic correlations estimated separately from data on the flies raised at 18 °C and 25 °C also were remarkably similar with none significantly different for the O'Hara and Trinkey populations. For Hemmant, two genetic correlations were significantly higher at 18 °C than at 25 °C, namely log (WL) and log (WP): 0.732 and 0.216 ($P < 0.01$), and log (WP) and log (WD): 0.134 and −0.425 ($P < 0.05$).

Discussion

Populations of *D. buzzatii* have differentiated genetically for body size and shape in the 55–60 years since their introduction into Australia (Barker et al. 1985), and this differentiation appears to be at least partly a response to different climatic regimes. Our results are largely consistent with and extend the work of Robertson (1987), who also studied the O'Hara and Hemmant populations used by us. An Australia-wide survey of *D. buzzatii* populations by Barker (unpublished) also corroborates these results by showing genetic variation in a number of size measures, as well as several environmental-tolerance and life-history traits.

Uniformly high estimates of heritability for size and shape measures were observed in all three populations and are generally consistent with previous estimates for these morphological traits in *Drosophila* species (Roff and Mousseau 1987). Phenotypic correlations among the measures were similar in all three populations and, as expected in characters thought not to be intimately connected to fitness, they were generally of the same sign and magnitude as their respective genetic correlations.

In the context of life-history evolution, and in particular the evolution of senescence, Clark (1987) suggested that genetic correlations may not be stable across environments. That is, where one or both of the traits is phenotypically plastic, the genetic correlation may change in magnitude or even in sign from one environment to another. Our results do not support this suggestion, in that the genetic correlations were generally not different, either among populations from different natural environments or between the different laboratory temperatures. Where there were significant differences among populations, they were essentially all between the coastal Hemmant population and the two inland populations, i.e., between the population where there was no evidence for genetic variation in plasticity (Hemmant) and the two other populations. Further, the only significant differences between laboratory temperatures were for two correlations in the Hemmant population. For the traits thorax length and wing length, Scheiner et al. (1991) also found stability of the genetic correlation across populations and temperatures in *D. melanogaster*. However, the general stability of genetic correlations recorded here and by Scheiner et al. (1991) should not be taken to mean that correlations cannot change across populations and environments. In both studies, the traits relate to body size, and stability might be expected because of developmental integration (Stearns et al. 1991).

Nevertheless, given this conclusion of a general stability of genetic correlations among these morphological traits, the significant differences noted above warrant further comment. In both cases where Hemmant differs from the other two populations, the genetic correlation involves proximal wing length [log (WP)]: (1) with log (TL) where the correlation for Hemmant is negative, but positive in the other populations, and (2) with log (W/T) where the correlation is positive in all three populations, but significantly larger for Hemmant. Further, for the two genetic correlations that were significantly different

between 18 °C and 25 °C in Hemmant, both involve log (WP).

The *Drosophila* wing develops from an imaginal disc that is divided into clonally separate anterior and posterior compartments, which are established very early during development. Later, at the beginning of the third larval instar, the wing blade is transversely subdivided in both anterior and posterior regions, into proximal and distal compartments (Garcia-Bellido et al. 1973). The proximal compartment includes about the first half of our proximal wing length measure and the distal compartment the remainder of the proximal measure and all of our distal wing length measure.

Previous studies of the quantitative genetics of wing size and shape that have considered the different developmental compartments (e.g., Cavicchi et al. 1985) have emphasized differences between the early established anterior and posterior compartments, but not between the proximal and distal. We can offer no explanation for the apparent differences in the genetic covariances involving proximal wing length in the Hemmant population. Clearly, a detailed study of the temperature effects and phenotypic plasticity of wing dimensions, separately measuring dimensions in the proximal and distal compartments, are necessary to clarify the developmental basis of wing size and shape variation.

However, given their origin in two developmental compartments, it is not surprising that our two wing length measures behave differently, with the significant temperature × sex interaction for wing length due primarily to distal wing length, with low or non-existent correlations between proximal and distal wing lengths (Table 5), and the variance due to temperature being much greater for the proximal measure (Table 4).

Why have these populations genetically differentiated in body size? Genetic drift following fragmentation of the range of *D. buzzatii* in Australia some 55–60 years ago is unlikely for several reasons. Firstly, all populations had high levels of additive genetic variation which would not be expected if any had been subjected to recent bottleneck events. Secondly, extensive allozyme data showed that populations have maintained considerable electrophoretic variation while differentiating, in some cases clinally, in allele frequencies (Sokal et al. 1987). Could the different mean phenotypes be due simply to an unfavorable correlation structure which keeps the populations from equilibrium (Via and Lande 1985), rather than to their having different phenotypic optima? We cannot answer this question directly. However, the 300 or more generations which have elapsed since the range fragmentation would most likely be sufficient to dismiss this explanation.

In these populations, body size was inversely related to the average annual temperature, i.e., flies from the warmer site (Hemmant, Av-T, Table 1) were smaller (Table 2) when raised under constant laboratory conditions. Similar observations were made by Tantawy and Mallah (1961) for both *D. melanogaster* and *D. simulans* along a transect from Lebanon to Uganda, and for *D. subobscura* in Britain and Europe (Prevosti 1955; MacFarquhar and Robertson 1963; Misra and Reeve 1964; Pfriem 1983). However, the situation is more complex than these results might suggest. Levins (1969), in the course of studying thermal acclimation in a number of *Drosophila* species, fround *D. melanogaster* to be genetically larger (heavier body weight) when sampled from hot coastal locations in Puerto Rico than when derived from cool upland populations. He hypothesized that the differences between his results and those of Tantawy and Mallah were a consequence of the different humidity regimes in the transects studied. In Puerto Rico, cool areas are wet, and hot areas are dry, while the opposite situation is found for Tantawy and Mallah's (1961) transect. The differences could be explained if adaptation for desiccation resistance was an important component of selection on adult body size. A hot, dry habitat would impose contradictory demands on body size. A higher temperature of development results in a shorter development time and hence a smaller body size, while desiccating conditions would select for larger adult body size (Levins 1969). Perhaps an analogous result was seen in *D. robusta*. Here, Stalker and Carson (1947) found that geographic variation in thorax length varied slightly positively, but significantly, with mean annual temperature, but that wing length showed a significant negative correlation with mean annual temperature. Altitudinal and seasonal variation in thorax and wing lengths, as well as other measures, showed negative relationships with temperature (Stalker and Carson 1948, 1949). Temperature, at least as we usually measure it, plainly is not a complete explanation of body size, though it is an important determinant.

The most interesting observation in the present study is the difference among populations in genotype-by-environment interactions for body size at different temperatures of development. That is, the populations differ in the amount of genetic variation for phenotypic plasticity of response to temperature. Flies from Hemmant, a coastal location which has a benign temperature regime both diurnally and seasonally, do not exhibit detectable genetic variation in the plasticity of response to variation in temperature of development. The inland populations, O'Hara and Trinkey, on the other hand, do show genetic variation in phenotypic plasticity, in that flies from these populations differ genetically in their abilities to attain a particular body size at a given temperature.

Is there a causal connection between the extent of temperature variation and the level of genetic variation in phenotypic plasticity? Schlichting and Levin (1990) note the difficulty of inferring relationships between environ-

mental variation and differences among populations in their plastic responses. They were concerned with the phenotypic expression of plastic responses, and we recognize the extra uncertainty in inferring a relationship to the level of genetic variation in plasticity. Plastic responses are not necessarily adaptive, and there are a variety of constraints on the evolution of plasticity (Bradshaw 1965; Via and Lande 1985; Schlichting 1986). However, we suggest that some variable temperature regimes could maintain increased genetic variation in the ability to attain a particular size. One can imagine that under some conditions the ability to maintain a particular body size despite temperature fluctuations would be advantageous, while under other conditions a more plastic response would be favored. It is plausible that these conflicting outcomes could be favored at different times in a variable environment. While considerable theoretical and empirical work deals with the effect of temporal variation on the maintenance of genetic variation (e.g., Hedrick 1976, 1986), little deals with the possibility that selection in a variable environment could be acting to maintain genetic variation in the degree of plasticity of response to that environment. Orzack (1985) investigated a single-locus model of the genetics of homeostasis which bears on this problem but it remains to be seen what the effects of polygenic inheritance would be. If phenotypic plasticity is treated as a trait with a heritable component, then models for the maintenance of quantitative genetic variation would be applicable. However, the maintenance of genetic variation in phenotypic plasticity appears to require separate consideration, as (1) the expression of plasticity depends on response to two or more environments, (2) temporal environmental heterogeneity selects for increased plasticity (Lynch and Gabriel 1987; Gabriel and Lynch 1992), and (3) temporal environmental heterogeneity may act to maintain genetic variation for plasticity, as our results suggest. Obviously, further observations of associations between levels of temporal variability in environments and the magnitudes of interaction between genotypes and environments are needed.

Acknowledgements. Professor Forbes Robertson provided the basis for this work and made valuable comments on early results. Tim Prout and Bill Etges provided helpful comments on an early version of the manuscript. We especially thank Sam Scheiner for a very helpful and constructive review of a previous version of the manuscript. Darren Schafer's good humoured help with field work is much appreciated. Don Fredline, Chris Leger, Annette Edmonds, and Jenny Druitt provided technical help and comic relief. Ron Lyons of the UNE Computer Centre provided invaluable assistance with RS-232 interfacing. This work was supported by a grant from the Australian Research Council to J.S.F.B.

References

Atchley WR, Gaskins CT, Anderson D (1976) Statistical properties of ratios. I. Empirical results. Syst Zool 25:137–148

Atkinson WD (1979) A comparison of the reproductive strategies of domestic species of *Drosophila*. J Anim Ecol 48:53–64

Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Adv Genet 13:115–155

Barker JSF, Mulley JC (1976) Isozyme variation in natural populations of *Drosophila buzzatii*. Evolution 30:213–233

Barker JSF, Sene FdeM, East PD, Pereira MAQR (1985) Allozyme and chromosomal polymorphism of *Drosophila buzzatii* in Brazil and Argentina. Genetica 67:161–170

Bowman JC (1972) Genotype × environment interactions. Ann Génét Sél anim 4:117–123

Cavicchi S, Guerra D, Giorgi G, Pezzoli C (1985) Temperature-related divergence in experimental populations of *Drosophila melanogaster*. I. Genetic and developmental basis of wing size and shape variation. Genetics 109:665–689

Clark AG (1987) Senescence and the genetic-correlation hang-up. Am Nat 129:932–940

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

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

de Jong G (1990b) Genotype-by-environment interaction and the genetic covariance between environments: multilocus genetics. Genetica 81:171–177

Dickerson GE (1962) Implications of genetic-environmental interaction in animal breeding. Anim Prod 4:47–63

Falconer DS (1989) Introduction to quantitative genetics, 3rd edn. Longman, Harlow, England

Falconer DS, King JWB (1953) A study of selection limits in the mouse. J Genet 51:561–581

Gabriel W, Lynch M (1992) The selective advantage of reaction norms for environmental tolerance. J Evol Biol 5:41–59

Garcia-Bellido A, Ripoll P, Morata G (1973) Developmental compartmentalisation of the wing disk of *Drosophila*. Nature 245:251–253

Gomulkiewicz R, Kirkpatrick M (1992) Quantitative genetics and the evolution of reaction norms. Evolution 46:390–411

Gupta AP, Lewontin RC (1982) A study of reaction norms in natural populations of *Drosophila pseudoobscura*. Evolution 36:934–948

Haldane JBS (1946) The interaction of nature and nurture. Ann Eugen 13:197–205

Harvey WR (1982) Mixed model capabilities of LSML76. J Anim Sci 54:1279–1285

Harvey WR (1988) User's Guide for LSMLMW. PC-1 Version. Copyright WR Harvey

Hedrick PW (1976) Genetic variation in a heterogeneous environment. II. Temporal heterogeneity and directional selection. Genetics 84:145–157

Hedrick PW (1986) Genetic polymorphism in heterogeneous environments: a decade later. Annu Rev Ecol Syst 17:535–566

Levins R (1969) Thermal acclimation and heat resistance in *Drosophila* species. Am Nat 103:483–499

Lynch M, Gabriel W (1987) Environmental tolerance. Am Nat 129:283–303

McFarquhar AM, Robertson FW (1963) The lack of evidence for co-adaptation in crosses between geographical races of *Drosophila subobscura* Coll. Genet Res 4:104–131

Misra RK, Reeve ECR (1964) Clines in body dimension in populations of *Drosophila subobscura*. Genet Res 5:240–256

Orzack SH (1985) Population dynamics in variable environments V. The genetics of homeostasis revisited. Am Nat 125:550–572

Pani SN, Lasley JF (1972) Genotype × environment interactions in animals. Res Bull 992. University of Missouri-Columbia, Missouri, USA

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

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

Pfriem P (1983) Latitudinal variation in wing size in *Drosophila subobscura* and its dependence on polygenes in chromosome O. Genetica 61:221–232

Prevosti A (1955) Geographical variability in quantitative traits in populations of *Drosophila subobscura*. Cold Spring Harbor Symp Quant Biol 20:294–299

Prout T, Barker JSF (1989) Ecological aspects of the heritability of body size in *Drosophila buzzatii*. Genetics 123:803–813

Reeve ECR, Robertson FW (1953) Analysis of environmental variability in quantitative inheritance. Nature 171:874–875

Robertson A (1959) The sampling variance of the genetic correlation coefficient. Biometrics 15:469–485

Robertson FW (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 FW (1987) Variation of body size within and between wild populations of *Drosophila buzzatii*. Genetica 72:111–125

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

Robertson FW, Reeve E (1955) Studies in quantitative inheritance. VIII. Further analyses of heterosis in crosses between inbred lines of *Drosophila melanogaster*. Z indukt Abstamm Vererblehre 86:439–458

Roff D (1977) Dispersal in dipterans: Its costs and consequences. J Anim Ecol 46:443–456

Roff DA, Mousseau TA (1987) Quantitative genetics and fitness: lessons from *Drosophila*. Heredity 58:103–118

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

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

Scheiner SM, Goodnight CJ (1984) The comparison of phenotypic plasticity and genetic variation in populations of the grass *Danthonia spicata*. Evolution 38:845–855

Scheiner SM, Lyman RF (1989) The genetics of phenotypic plasticity I. Heritability. J Evol Biol 2:95–107

Scheiner SM, Caplan RL, Lyman RF (1991) The genetics of phenotypic plasticity. III. Genetic correlations and fluctuating asymmetries. J Evol Biol 4:51–68

Schlichting CD (1986) The evolution of phenotypic plasticity in plants. Annu Rev Ecol Syst 17:667–693

Schlichting CD, Levin DA (1990) Phenotypic plasticity in *Phlox*. III. Variation among natural populations of *P. drummondii*. J Evol Biol 3:411–428

Sokal RR, Oden NL, Barker JSF (1987) Spatial structure in *Drosophila buzzatii* populations: simple and directional spatial autocorrelation. Am Nat 129:122–142

Stalker HD, Carson HL (1947) Morphological variation in natural populations of *Drosophila robusta* Sturtevant. Evolution 1:237–248

Stalker HD, Carson HL (1948) An altitudinal transect of *Drosophila robusta* Sturtevant. Evolution 2:295–305

Stalker HD, Carson HL (1949) Seasonal variation in the morphology of *Drosophila robusta* Sturtevant. Evolution 3:330–343

Starmer WT, Barker JSF (1986) Ecological genetics of the *Adh-1* locus of *Drosophila buzzatii*. Biol J Linn Soc 28:373–385

Starmer WT, Wolf LL (1989) Causes of variation in wing loading among *Drosophila* species. Biol J Linn Soc 37:247–261

Stearns SC (1989) The evolutionary significance of phenotypic plasticity. BioScience 39:436–445

Stearns S, de Jong G, Newman B (1991) The effects of phenotypic plasticity on genetic correlations. Trends Ecol Evol 6:122–126

Tantawy AO, Mallah GS (1961) Studies on natural populations of *Drosophila*. I. Heat resistance and geographical variation in *Drosophila melanogaster* and *D. simulans*. Evolution 15:1–14

Via S (1984) Th quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and among host plants. Evolution 38:896–905

Via S, Lande R (1985) Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505–522

Wilkinson GS (1987) Equilibrium analysis of sexual selection in *Drosophila melanogaster*. Evolution 41:11–21

Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice-Hall Inc, Englewood Cliffs, New Jersey