Heritability and Selection on Body Size in a Natural Population of *Drosophila buzzatii*

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ABSTRACT

An attempt was made to assess whether the phenotypic differences in body size (as measured by wing length) between wild-caught mating and single *Drosophila buzzatii* males could be attributed to genetic differences between the samples. Mating males were found to be larger and less variable than a random sample of the population. The progeny of the mating males (produced by crossing to a random female from a stock derived from the same population) were on average larger than those of the single males, but not significantly so ($P = 0.063$), and less phenotypically variable. This difference in variance between the samples suggests that there are indeed genetic differences between the paternal samples but tests for significant differences in the additive genetic component of variance proved inconclusive. For both samples it was found that while the ratio of additive genetic variation in the laboratory to phenotypic variation in the field yielded estimates of $\hat{h}^2_{s(N)} \simeq 10\%$ the regression of offspring reared in the laboratory on parents from the wild was not significantly different from zero. In addition, it was found that the average development time of the progeny of the mating males is shorter than that of the random sample.

THE apparent simplicity of the basic theory of natural selection may lead one to suppose that the study of its action in nature would be equally simple. Unfortunately, neither the theory of natural selection, nor the techniques required for its study in nature are as straightforward as they at first appear. Consider for example the case of selection on body size in the cactophilic species *Drosophila buzzatii*. Positive phenotypic correlations between body size (as measured by thorax length) and three fitness components (longevity, fecundity and mating success) have been measured in a natural population (SANTOS *et al.* 1988, 1992b). Similar correlations are seen in many other species (see ROFF 1992 for a review). There is also evidence for the presence of significant amounts of additive genetic variation for body size both in the laboratory (ROBERTSON 1987; RUIZ *et al.* 1991; THOMAS and BARKER 1993) and in natural populations of *D. buzzatii* (PROUT and BARKER 1989; RUIZ *et al.* 1991). A simple interpretation of these findings leads one to one of the following conclusions: that the population is not at an evolutionary equilibrium and that the mean body size is increasing with time, or that if the population is indeed at equilibrium, then the positive selection for large size during the adult stage must be counterbalanced by selection in some other part of the life cycle, *i.e.*, there exists a "trade off" between opposing selective forces.

Artificial selection for large adult body size results in an increase in larval development time in *D. melanogaster* (ROBERTSON 1960; PARTRIDGE and FOWLER 1993; SANTOS *et al.* 1994), with a consequent drop in larval viability under high densities (SANTOS *et al.* 1992a; PARTRIDGE and FOWLER 1993). There is a demographic advantage to be gained by early breeding, especially during periods of population expansion (COLE 1954; LEWONTIN 1965). Overall, it appears that body size in Drosophila may reflect an evolutionary compromise between the effects of genetic variation on larval and adult performance (see also WILKINSON 1987). It is not at all obvious, however, that the phenotypic correlation between male size and fitness components found in the field produces a genetic response. Despite the presence of heritable variation in body size there are two reasons why the response to selection may not be that predicted by the simple selection equation $R = h^2 S$. One is the effect of selection on other, correlated characters (LANDE and ARNOLD 1983), and the other is the effect of environmental correlations between the trait being studied and fitness (PRICE *et al.* 1988; KIRKPATRICK *et al.* 1990; RAUSHER 1992; VAN TIENDEREN and DE JONG 1994). Although the phenotypic effect of natural selection on any particular trait (*i.e.*, the change in phenotypic distribution within a generation) is only dependent on the selection applied and the phenotypic distribution in the population, the immediate genetic response depends upon, in addition to the heritability and fitness function of the trait in question, the heritabilities and genetic correlations between the trait of interest and all other traits that are affected by selection. In most studies, only one or a few traits are investigated. Because the number of traits that could potentially be

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affected by selection is very high, it is in practice impossible to measure all of the parameters necessary for an accurate prediction of the short term response to selection (BARTON and TURELLI 1989).

Even if one were certain that only the traits being studied were under selection, the phenotypic correlation between the trait and fitness would not necessarily provide any information on the genetic correlation between the two required for the prediction of the selection response (RAUSHER 1992; VAN TIENDEREN and DE JONG 1994). For example, environmental correlations as a result of variation in nutritional condition have been invoked to explain the stability of breeding date in birds in the face of strong directional selection and ample heritable variation (PRICE *et al.* 1988; KIRKPATRICK *et al.* 1990).

Here, we attempt to assess whether or not there is a genetic correlation underlying one of the phenotypic correlations seen between body size and one of the fitness components (namely male mating success) in *D. buzzatii* populations. Male mating success was chosen, rather than fecundity or longevity, because of these three it is by far the easiest to measure and because, of the total phenotypic selection seen on body size, 60% can be attributed to variation in mating success (SANTOS *et al.* 1992b). If the differences seen between samples of mating and single males represent genetic differences (that are not cancelled out by selection on other, unmeasured characters), we expect to see corresponding differences between their progeny after crossing to a random female and rearing in standard conditions. In particular, if selection does evoke a genetic response, we expect to see that the progeny of mating males are larger and less variable than those of the random sample. In addition to estimates of the mean body size (measured as wing length) of the progeny of the two samples, estimates of the heritability of this character in the natural and laboratory environments are obtained. Because it is suggested that there is a trade off between selection on body size and on development time, the development time of the progeny was also measured.

MATERIALS AND METHODS

Samples: Samples were collected from a population in an abandoned *Opuntia ficus-indica* plantation at Carboneras (Almería, S.E. Spain) (RUIZ *et al.* 1986). Flies were collected between 1800 and 2030 hours on each of 7 days between September 12 and 18, 1993. Rotting Opuntia cladodes were found and opened to attract flies. Mating pairs were collected using an insect aspirator, as well as a similar-sized sample of single males. Only those pairs that remained *in copula* for >20 sec were collected. The females from each pair were discarded and the males kept in vials (2 × 8 cm) containing 6 ml of standard cornmeal-agar-yeast food. A total of 124 single males (S males) and 116 mating males (P males) were collected.

Crosses: On September 19, wild males (105 S and 105 P) were individually crossed to three well-fed virgin females between 4 and 5 days old (F_3 females from an outbred laboratory stock derived from 21 females collected from the same population on July 29). Flies were left to mate for 48 hr after which the males were fixed in a 3:1 mixture of alcohol and glycerol and each female transferred to a vial with fresh food and left to lay eggs for 16 hr and then discarded. Emerging progeny were removed from the vials and fixed in alcohol/glycerine every 24 hr. For the first 9 days of the experiment, all vials were kept at room temperature (22–24°) in the makeshift laboratory near the field site until our return to Barcelona after which they were maintained at 23° under a 12:12 light/dark cycle with uncontrolled humidity. Throughout the experiment the vials were kept together in groups of six, each consisting of the three vials from one mating male (P) and one non mating (S) male.

Traits studied: Wing length was measured from the proximal junction of 4th and 5th longitudinal veins to the intersection of the third longitudinal vein with the distal wing margin (DEMEREC 1965). Wings were removed and fixed in DPX under coverslips on microscope slides, and viewed under ×32 magnification (1 pixel = 0.00498 mm) using the VICOM Digital Image Processor at the Centre de Tractament d'Imatges de la Universitat Autònoma de Barcelona (U.A.B.). From the image of the wing projected onto a video screen the coordinates of the two reference points were recorded using a "mouse" as an interactive device. One wing was measured for each of the wild-caught males except two from each sample which had been damaged. Two random sons from each female that produced two or more male progeny were measured.

Development time was estimated as the number of days from the midpoint of the 16 hr for which the females were ovipositing to adult emergence, where all flies counted at a particular scoring were taken as having emerged at the midpoint in time between that scoring and the previous one.

Estimation of variance components and heritabilities: Because not all females produced offspring, the final data set for wing length of the progeny was an unbalanced two-way nested classification for S (46 with three females, 35 with two, and 18 with one; $N = 452$ individuals measured) and P males (54 with three females, 35 with two, and eight with one; $N = 480$). All the data for wing length were $\log_e$-transformed. The model of analysis is

$$y_{ijk} = \mu + \alpha_i + \delta_{j(i)} + \beta x_{ij} + e_{ijk}, \qquad (1)$$

where μ is the overall grand mean, α_i is the random effect of the ith male (sire), $\delta_{j(i)}$ is the random effect of the jth female (dam) within the sire i, x_{ij} is the number of offspring of the jth dam with ith sire and β is the coefficient of the covariate x, and e_{ijk} is the residual error associated with the $\log_e$(wing length) of the ijkth individual. The number of offspring per female needed to be incorporated into the analysis as a covariate because the larval density was not strictly controlled. The x_{ij} values may account for a possible increased covariance between half- and/or full-sibs due to environmental correlations.

Least-squares (ANOVA) estimates of variance components were obtained for each sample. The data were first fitted to the linear model

$$y_{ijk} = \beta_0 + \beta x_{ij} + \epsilon_{ijk}, \qquad (2)$$

and the residuals (ϵ_{ijk}) analyzed following the formulae for the two-way nested classification given in SEARLE *et al.* (1992, pp. 429–430). No correction was made for the degree of freedom lost in estimating β. Restricted maximum likelihood (REML) estimates of model 1 were also obtained for each sample using the program 3V of the BMDP Statistical Software package (1992), implemented on a VAX-6610 VMS at the Centre de Càlcul de la U.A.B. Both ANOVA and REML estimates were very similar. However, because each REML esti-

mate of Equation 1 takes ~20 min of CPU time, and delete-one jackknife data resampling was done to estimate variance components for wing length (see below), we will only provide the ANOVA estimates.

The regression of $\log_e$(wing length) of laboratory offspring on male parents from nature was estimated using the linear model

$$y_{ijk} = \beta_1 + \beta_{(O_L,F_N)}z_i + \beta x_{ijk} + \xi_{ijk}, \quad (3)$$

were z_i is the $\log_e$(wing length) of the ith sire, y_{ijk} and x_{ij} are as defined above and ξ_{ijk} the residuals.

Estimates of the genetic variances and their standard errors were obtained following BECKER (1984). For each sample, several estimates of the heritability of wing length were made. Heritability in the laboratory environment was estimated from the covariance between half $[\hat{h}^2_{s(L)} = (4\hat{\sigma}^2_\alpha)/(\hat{\sigma}^2_\alpha + \hat{\sigma}^2_\delta + \hat{\sigma}^2_\varepsilon) = V_{A,s(L)}/V_{P(L)}]$ and full-sibs $[\hat{h}^2_{d(L)} = 2(\hat{\sigma}^2_\alpha + \hat{\sigma}^2_\delta)/(\hat{\sigma}^2_\alpha + \hat{\sigma}^2_\delta + \hat{\sigma}^2_\varepsilon) = V_{A,d(L)}/V_{P(L)}]$ (a caret denotes an estimator of a parameter). Following LANDE (Appendix to COYNE and BEECHAM 1987, p. 729) and RISKA *et al.* (1989), three estimates of the narrow sense heritability in nature were made. The ratio of the additive genetic variance estimated in the laboratory as $4\text{Cov}_{\text{half-sibs}}$ to the phenotypic variance estimated in the field $[\hat{h}^2_{s(N)} = (V_{A,s(L)}/V_{P(N)}]$ gives an estimate of the natural heritability based on the assumption that additive genetic variance does not differ between the laboratory and field environments. The regression of laboratory reared sons on wild-caught fathers $(2\hat{\beta}_{(O_L,F_N)})$ gives a second estimate that assumes no genotype-environment interaction (defined as no difference in additive genetic variance and perfect correlation of the expression of genotypes in the two environments) (PROUT and BARKER 1989, p. 808). The third estimate is $(V_{P(N)}/V_{A,s(L)})(2\hat{\beta}_{(O_L,F_N)})^2 = \hat{\gamma}^2\hat{h}^2_N$, which is a lower bound for the natural heritability and is available whenever the additive genetic variance in the laboratory is >0 but is an underestimate of natural heritability whenever the additive genetic correlation across environments (γ) is <1 (RISKA *et al.* 1989). Rough estimates of the variance were calculated as

$$\begin{aligned}\text{Var}(\hat{\gamma}^2\hat{h}^2_N) &= \text{Var}\left[\frac{V^2_{P(N)}}{V^2_{A,s(L)}}(2\hat{\beta}_{(O_L,F_N)})^2\right] \\ &\approx 16[\text{Var}^2(\hat{\beta}_{(O_L,F_N)}) + 4\hat{\beta}^2_{(O_L,F_N)}\text{Var}(\hat{\beta}_{(O_L,F_N)})] \\ &\times\left[\left(\frac{V_{P(N)}}{V_{A,s(L)}}\right)^2 + \frac{V^2_{A,s(L)}\text{Var}(V_{P(N)}) + V^2_{P(N)}\text{Var}(V_{A,s(L)})}{V^4_{A,S(L)}}\right] \\ &+ 16\hat{\beta}^4_{(O_L,F_N)}\frac{V^2_{A,s(L)}\text{Var}(V_{P(N)}) + V^2_{P(N)}\text{Var}(V_{A,s(L)})}{V^4_{A,s(L)}}, \quad (4)\end{aligned}$$

but is unlikely to be particularly accurate because it assumes that there is no covariance between any of the variance components.

The difference between the average $\log_e$(wing length) of the offspring of S and P males was tested by the standard ANOVA F-value based on a mixed model similar to Equation 1 with sample added as a fixed effect and REML estimates of variance components.

ANOVA estimates of variance components for log(development time) were obtained for each sample by adjusting the model

$$y_{ij} = \mu + \alpha_i + \beta x_{ij} + e_{ij}, \quad (5)$$

where μ is the overall grand mean, α_i is the random effect of the ith sire, x_{ij} is the number of offspring of the jth dam with ith sire and β is the coefficient of the covariate x, and e_{ij} is the residual error associated with the log(development time) of the ijth vial. The difference between the average log(development time) of the offspring of S and P males was tested in a similar way as has been previously described for wing length.

RESULTS

Phenotypic selection and response: Summary statistics are given in Table 1. No deviation from normality was detected in the distributions of $\log_e$(wing length) in the two samples of sires (Kolmogorov-Smirnov tests. S males: $D = 0.052$, $P > 0.05$; P males: $D = 0.039$, $P > 0.05$) nor of their progeny (S males: $D = 0.044$, $P > 0.05$; P males: $D = 0.023$, $P > 0.05$). For the parental generation, there is a clear difference in the distribution of wing length between the two samples. Wing length of S males has a lower mean and higher variance than that of P males. These differences translate to a standardized selection differential of $i = 0.36$, and to a proportional change in the variance of $j' = -0.25$ (ENDLER 1986, pp. 171–173).

Mean wing length of the progeny of S males is slightly smaller than that of P male progeny but not significantly so ($P = 0.06$). As in the parental samples, variance in wing length is significantly lower in the progeny of P males than S males.

The average number of offspring per productive female was approximately equal for both samples. However, there was a relatively high variability in the number of flies per vial, which resulted in a significant negative regression between average wing size of progeny against the number of offspring per family. REML estimates of regression coefficients from model 1 were $\hat{\beta} = -0.000808$ ($P < 0.001$) and $\hat{\beta} = -0.000711$ ($P < 0.001$), for the progenies of S and P males, respectively. The two regression slopes are not significantly different ($F_{[1, 928]} = 0.558$, $P = 0.455$). However, the significant regression justifies the inclusion of the number of offspring per female as a covariate in the estimation of variance components.

Development time is approximately normally distributed on a log scale in the progeny of P males ($D = 0.069$, $P > 0.05$) but not in those of S males ($D = 0.146$, $P < 0.01$). No significant regression of average development time on number of offspring per family is observed for either sample (ANOVA regression slope estimates from model 5 are: $\hat{\beta} = -0.000237$, $P = 0.117$ and $\hat{\beta} = 0.000003$, $P = 0.966$ for S and P males, respectively). Mean development time is ~5 hr less for the progeny of P males than for the progeny of S males. The variance in development time is also significantly less in the P male sample (Table 1). Because the F test is very sensitive to departures from normality, we performed a sampled randomization test to empirically obtain the distribution of variance ratios (SOKAL and ROHLF 1981, pp. 791–795). Only 61 out of 5000 ($P = 0.012$) random partitions were more deviant than the observed F value, supporting the hypothesis that devel-

TABLE 1

Between single males (S) and mating males (P) comparisons

	S males	P males	F^a	P
Sires				
Wing length (mm)	1.893 ± 0.117	1.936 ± 0.092	8.73 ± 1.73	0.004 ± 0.003
n	103	103		
Offspring				
Wing length (mm)	2.201 ± 0.060	2.210 ± 0.052	3.45 ± 1.37	0.063 ± <.001
n	452	480		
No. of offspring per fertile female[b]	24.248 ± 13.118	24.146 ± 12.296	0.01 ± 1.14	0.931 ± 0.162
n	226	240		
Mean development time[c] (days)	15.420 ± 0.846	15.211 ± 0.434	7.82 ± 3.79	0.005 ± <.001
n	225	240		

Values are means ± SD.

[a] F ratios for average comparisons of offspring $\log_e$(wing length) and log(developmental time) based on asymptotic variance-covariance matrix (REML method). Standard one-way ANOVAs were carried out in $\log_e$(wing length) of sires and number of offspring per fertile female. F-test for the variance ratio of offspring wing length based on the estimate of $V_{P(L)}$ in Table 3.

[b] Covariate x_{ij} in model 1.

[c] Development time not recorded for one vial of S male progeny.

opmental time is indeed less variable in the offspring of P males.

Variance components and heritability estimates; wing length: Variance components (between sires, between dams, and error) are given in Table 2. Because the number of sons measured per female was always two, the ordinary F-test of effects due to sires (σ^2_a) provides an exact test of significance for the additive genetic variance component in the laboratory environment ($F_{[98,\ 127]} = 1.490$, $P = 0.017$ for S males; $F_{[96,\ 143]} = 1.527$, $P = 0.011$ for P males) (SOKAL and ROHLF 1981, pp. 293–308). However, because of the heterogeneity of within family (sires) variances (see below), the biological conclusions to be drawn from these tests must be checked. MITCHELL-OLDS and BERGELSON (1990) have shown that delete-one-sire-family jackknife data resampling provides a robust test of significance to detect the genetic components of variance when there is heterogeneous within-family variances.

Table 2 also gives the jackknife estimates of variance components. For both samples, there is significant variation between sires and between dams. In both samples, the variance between dams is greater than between sires suggesting that there may be dominance and/or maternal effects on wing length (large maternal effects are unlikely because all females were grown under controlled conditions). Dominance and maternal variances were estimated as $\hat{\sigma}^2_{dm} = \hat{\sigma}^2_\delta - \hat{\sigma}^2_a$ (MITCHELL-OLDS and BERGELSON 1990), which is statistically significant for S males (Table 2).

Estimates of the components of genetic variation and heritabilities are shown in Table 3. All laboratory based estimates of variance components and heritabilities are greater for the unmated than for the mated males, sug-

TABLE 2

ANOVA estimates of variance components for wing length

Variace component	Direct estimate	Jackknife estimate	Upper and lower 95% limits		Lower (one-tailed) 95% limit
S males					
σ^2_a	0.9021	0.9015	1.7491	0.0540	0.1923
σ^2_δ	2.9028	2.9020	4.1973	1.6068	1.8182
σ^2_{dm}	2.0007	2.0192	3.8858	0.1527	0.4573
σ^2_e	2.5869	2.5996	3.2910	1.9083	2.0211
P males					
σ^2_a	0.5754	0.5753	1.1213	0.0294	0.1185
σ^2_δ	1.3265	1.3270	1.9714	0.6826	0.7878
σ^2_{dm}	0.7510	0.7516	1.7051	−0.2018	−0.0461
σ^2_e	2.7486	2.7492	3.5194	1.9789	2.1047

σ^2_a, σ^2_δ, σ^2_{dm}, and σ^2_e represent components of variance attributable to sires, dams, dominance and/or maternal effects, and within family variation, respectively. Jackknife estimates are from an unweighted, delete-one-sire family jackknife. Confidence limits were obtained from normal-approximation jackknife interval estimators (SOKAL and ROHLF 1981, pp. 795–799). Wing length is in $(\log_e \text{mm})^2 \times 10^4$.

TABLE 3

ANOVA estimates of genetic variance components for wing length and heritabilities

Component	S males	P males
$V_{A,s(L)}$	3.6084 ± 1.5420	2.3018 ± 0.9088
$V_{A,d(L)}$	7.6097 ± 0.8888	3.8038 ± 0.5894
$V_{P(L)}$	6.3918 ± 0.4444	4.6505 ± 0.2947
$V_{P(N)}$	40.0181 ± 5.5495	23.1487 ± 3.2102
$\hat{h}^2_{s(L)}$	0.565 ± 0.238	0.495 ± 0.193
$\hat{h}^2_{d(L)}$	1.191 ± 0.081	0.818 ± 0.102
$\hat{h}^2_{s(N)}$ [a]	0.090 ± 0.039	0.099 ± 0.039
$2\hat{\beta}_{(O_L,F_N)}$ [b]	−0.061 ± 0.037	0.061 ± 0.040
$\hat{\gamma}^2\hat{h}^2_N$	0.041 ± 0.061	0.037 ± 0.058

Wing length is in $(\log_e \text{mm})^2 \times 10^4$; heritibilities are from model 1. Values are means ± SE.

[a] Standard error obtained as $\sqrt{\frac{\text{Var}(V_{A,S(L)})}{V^2_{P(N)}}}$.

[b] From 97 males S and 95 P.

gesting that the lower phenotypic variance of the mating males does indeed reflect lower genetic variance. Statistical comparison of additive genetic variances between the samples can be carried out by using the Bonferroni method (RICE 1989) or the likelihood ratio test (SHAW 1991; SHAW and BILLINGTON 1991).

The *F*-ratios for the phenotypic and additive variances of the offspring of S and P males were 1.374 ($P < 0.001$) and 1.568 ($P = 0.014$), respectively. Given that both *P*-values are <0.025, it seems that a real reduction in both variance components as a result of the phenotypic selection for wing length in nature has taken place. However, this conclusion is not reached when the likelihood ratio test for the null hypothesis H_0: $V_{A(\text{S males})} = V_{A(\text{P males})}$ (V_A is the additive genetic variance), with an unconstrained REML analysis (*i.e.*, variance estimates are not confined to the parameter space and can be negative) using the residuals from model 2, is applied ($\chi^2_{[1]} = 0.386$, $P = 0.535$). Although both approaches are conservative, it is not obvious what biological conclusion can be drawn from these contradictory results.

Laboratory estimates of narrow sense heritability ($\hat{h}^2_{s(L)}$) are quite high for both S and P males. The amount of phenotypic variation in the field is between ~5 (P males) and 6.25 (S males) times greater than in the laboratory environment, and so the estimates of natural heritability based on the ratio of additive genetic variation in the laboratory to phenotypic variation in the field ($\hat{h}^2_{s(N)}$) are correspondingly smaller (~10% for both S and P males). Because $V_{A,s(L)} \geq 0.2160$ for S males and $V_{A,s(L)} \geq 0.1176$ for P males (Table 2), approximate lower limits of $\hat{h}^2_{s(N)}$ are ~0.005 in both samples.

The regression of laboratory reared offspring on wild-caught fathers is not significantly different from zero for both S ($t_{[441]} = -1.630$, $P = 0.104$) and P males ($t_{[469]} = 1.514$, $P = 0.131$) and therefore the estimates of natural heritability ($2\hat{\beta}_{(O_L,F_N)}$) are also nonsignificant. Although the increased covariance between half- and/or full-sibs due to differences in larval densities across vials was removed from the analysis, the vials were not kept in a temperature regulated incubator for the first 9 days of the experiment and variation in temperature [which is known to affect body size (PARSONS 1961; TANTAWAY and MALLAH 1961; ROBERTSON 1987)] across the trays of vials may have generated environmental covariation between siblings. This could explain the difference between the heritability estimates based on offspring-parent regression and additive genetic variance in the laboratory. The presence of such environmental covariation was tested for by looking at the correlation of average wing length between the S male and P male progeny per group. Because the males were paired at random any such correlation can only be as a result of environmental covariance. No significant correlation was observed ($r = 0.007$, $N = 91$, $P = 0.950$).

We are left with two possible (nonmutually exclusive) explanations for the difference between the heritability estimates: that the amount of additive genetic variance in the laboratory differs from that in nature or that the cross environment correlation (γ) is small. We find that for both samples $\hat{h}^2_{s(N)} > |2\hat{\beta}_{(O_L,F_N)}| > \hat{\gamma}^2\hat{h}^2_N$, so the lower bound $\hat{\gamma}^2\hat{h}^2_N$ (~4%) is the smallest of the three approximations. Either $\hat{h}^2_{s(N)}$ (~10%) or $|2\hat{\beta}_{(O_L,F_N)}|$ (~6%) could be closer to the true value of the natural heritability but we have no way of knowing if this is the case.

Variance components and heritability estimates; development time: Only laboratory estimates of the heritability of development time are available because we have no information on variation in developmental time in nature. There is significant variation in development time between sires for both samples (S males: $F_{[98,125]} = 1.694$, $P = 0.003$; P males: $F_{[96,142]} = 2.377$, $P < 0.001$). Estimates of variance components are shown in Table 4. Heritability estimates based on the uncorrected between sires variance components ($\hat{h}^2_{s(L)}$) are very high for both samples (>90%). However, strong correlation of average offspring development time between the S and P males in each group ($r = 0.228$, $N = 93$, $P = 0.028$) suggests that this value may be inflated due to effects of "environmental" covariance. This is likely to be more due to our experimental procedure than to genuine environmental effects on development time. During the few days of peak emergence it took >4 hr to empty all the vials and count the offspring. There was therefore a difference of ≥4 hr in the actual time at which the flies were counted between the first and the last group of vials (1 P male and 1 S male) during which the flies could have continued to emerge. This covariance in the actual time of counting generates an apparent covariance in development time.

TABLE 4

ANOVA estimates of genetic variance components for development time and heritabilities

Component[a]	S males	P males
$V_{A,S(L)}$	4.3676 ± 1.7023	2.1044 ± 0.5493
$V_{A,S(L\text{-}CE)}$[b]	2.7996	0.5364
$V_{P(L)}$	4.6641 ± 0.4528	1.4705 ± 0.1457
$V_{P(L\text{-}CE)}$	4.2721	1.0785
$h^2_{s(L)}$	0.936 ± 0.334	1.431 ± 0.297
$h^2_{s(L\text{-}CE)}$	0.655	0.497

$V_{A,S(L\text{-}CE)} = 4(\hat{\sigma}^2_a - \mathrm{Cov}_{CE})$; $V_{P(L\text{-}CE)} = \hat{\sigma}^2_a + \hat{\sigma}^2_e - \mathrm{Cov}_{CE}$. Development time is in (log days)$^2 \times 10^4$; heritabilities are from model 5. Values are means ± SE.

[a] To estimate the standard error of variance components the coefficients of MS_{sires} were calculated following SOKAL and ROHLF (1981, p. 214).

[b] Common environment (*CE*) estimated from the covariance between paired vials of S and P males (see text for details).

Note that because the progeny of one S male and one P male were always counted at the same time, this procedure cannot have affected the average development time of the two groups. The variance components were corrected for this effect by subtracting the covariance due to common environment (Cov_{CE}) of development time from the between sires component and from the total variance given by the ANOVA estimates. Cov_{CE} was estimated as the covariance of the residuals of the regression of log(development time) on number of offspring between the progeny of S and P males (Cov_{CE} = 0.0000392). Making the correction yields much lower, but still high, estimates of the heritability of development time (65% for S males, 48% for P males).

Genetic correlations: Significant laboratory estimates of the heritabilities of the two traits allow estimates of the genetic correlation between them to be made (BECKER 1984, pp. 113–117). Variation between sires for mean cross-products was statistically significant for S ($F_{[98,\,125]} = 7.11$; $P < 0.001$) but not for P males ($F_{[96,\,142]} = 0.79$; $P = 0.894$). The additive genetic correlation between the traits for S males was $\hat{r}_A = -0.0246$.

Major gene analysis of wing length: If all of the genetic variation measured were due to the action of polygenes of minor effect, one would expect to see homogeneity of within family variances. Deviation from this model was tested using the Bartlett test on the residuals of the regression of wing length against number of offspring and can be rejected for both S males ($\chi^2_{[98]} = 180.78$, $P < 0.001$) and P males ($\chi^2_{[96]} = 151.00$, $P < 0.001$). This test provides only weak evidence for the presence of major gene effects (LE ROY and ELSEN 1992) but is consistent with what is already known about the effect of inversions on size in the Carboneras population (RUIZ *et al.* 1991).

DISCUSSION

If body size in natural Drosophila populations reflects an evolutionary compromise between the conflicting effects of genetic variation on larval and adult performance, as was implied in previous laboratory studies (WILKINSON 1987; SANTOS *et al.* 1992a, 1994; PARTRIDGE and FOWLER 1993), the strongest evidence for a tradeoff between adult size and juvenile mortality would be the observation that mean wing length for the progeny of mated males was significantly larger than the mean wing length of the progeny of un-mated males.

The offspring of our mating male sample were slightly larger than those of the S males but not significantly so ($P = 0.06$, two-tailed test). If we assume only additive effects and that wing length is the only target of selection, the expected difference between the progeny samples is given by $R = {}^1/_2 h^2_N S\gamma$ (${}^1/_2$ because selection in our samples acts only on males), which is obviously dependent on the heritability of wing length. In the laboratory, we found substantial heritability for wing length similar to that previously found for thorax length (ROBERTSON 1987; PROUT and BARKER 1989; RUIZ *et al.* 1991; SANTOS *et al.* 1992b). To estimate the expected selection response, we need to know the heritability in the field but, because relatives cannot be identified in nature, only indirect estimates of this crucial parameter can be made (RISKA *et al.* 1989). We found that $\hat{h}^2_{s(N)} > |2\hat{\beta}_{(O_L,F_N)}| > \hat{\gamma}^{\,2}\hat{h}^2_N$ for both samples (Table 3). Even if we take $\hat{h}^2_{s(N)}$ as the closest approximation to the true natural heritability, little more than that the value of this parameter is non-zero (*i.e.*, $h^2_N \geq 0.005$ for both S and P males) can be said which, given the significant heritability in the laboratory, is not very surprising. Assuming that $\hat{h}^2_{s(N)}$ (Table 3) is the natural heritability of wing length for the S males and using the difference in means between the paternal sample, the expected difference between the progeny samples is only $R = 0.0019$. The actual difference observed is greater than this but nonsignificant. Obviously the probability of detecting significant differences of such small magnitudes is extremely small. A fundamental problem in our approach is that we look at the effect selection of parents in one environment (the field) has on their progeny in another (the laboratory). From our data we cannot exclude the possibility that there is in fact a strong genetic correlation between size and mating success and that natural heritability is much higher than our highest estimate but we see no differences between the sample means because of a low cross-environment correlation of wing length.

Although *D. buzzatii* is known to breed on discrete resources (BARKER 1977; SANTOS *et al.* 1989; THOMAS and BARKER 1990), our analysis was carried out only at the level of the population as a whole. PROUT and BARKER (1989, 1993) performed an analysis of the heritability of body size (thorax length) in *D. buzzatii* looking at variation within patches as well as in the population as a whole. They found substantial within- and between-rots additive genetic variation, and their

"across rots" estimate of heritability ($h^2 = 0.0595$; *i.e.*, the heritability of a natural population coming from different rots), would apply to a random mating population such as that in Carboneras (QUEZADA-DÍAZ *et al.* 1992; BARBADILLA *et al.* 1994). The lower bound estimate of V_A (*i.e.*, the additive genetic variance in the laboratory environment) in PROUT and BARKER (1989, p. 809) provides a population lower bound estimate, $h^2 < 0.041$, which happens to be identical to the lower bound for S male progeny (Table 3).

As well as changing character means, directional selection is expected to reduce genetic variances (FALCONER 1981; BULMER 1980). Although the cross-environment correlation may affect the magnitudes of the progeny sample variances, it should not affect the interpretation of the differences in variance between the samples. We find the progeny of P males to be significantly less phenotypically variable than the progeny of the S males, which seems to reflect the difference in phenotypic variance between the paternal samples. Apart from the unlikely possibility of nongenetic paternal effects, this difference can only be attributed to genetic differences between the paternal samples because all progeny were reared in standard conditions. We therefore do have evidence that there is a genetic component to the difference between the paternal samples. This difference in phenotypic variance may be attributed to differences in the additive and/or dominance components of variance (Tables 2 and 3). Estimates of the "causal" components of variance [*e.g.*, additive genetic variance (V_A), dominance (V_D) and environmental (V_E)] from the unconstrained REML analyses (see above) are, respectively, 3.6415, 7.9160, and −5.1707 for the S males and 2.3876, 2.8275, and −0.5659 for the P males. Tests for differences in the additive genetic component between our two samples yielded inconclusive results. A significant difference was detected using standard *F*-tests but not with the likelihood-ratio test. A problem with this test is that its power is low, especially for unconstrained analyses (SHAW 1991).

Dominance effects (V_D) are statistically significant in the S males only (Table 2). This is in agreement with the previously estimated contributions of the second and fourth chromosome inversions to the phenotypic variance for thorax length in wild flies from the same population (RUIZ *et al.* 1991). In this study, most of the phenotypic variance between karyotypes was explained by the linear regression on chromosome dose in the mating males whereas in the solitary males the proportion was much lower. It is possible that in the S males a significant proportion of the total genetic variance is dominance variance contributed by recessive or partially recessive deleterious mutations with pleiotropic side effects on body size. However, if quantitative variation is maintained as the pleiotropic side effect of deleterious mutations with various effects on the trait and fitness, only a small proportion of the total genetic variance is expected to be attributable to dominance variance (CABALLERO and KEIGHTLEY 1994). It would appear, therefore, that the value of V_D in S males is too high for genetic variance for body size in *D. buzzatii* to be explained by pleiotropic mutation models. Of course, dominance variance in the field could be much lower than that estimated in the laboratory.

The expected change in variance over one generation depends on the nature of the selection gradient and the genetics of the trait in question. Under the infinitesimal model, in a large random mating population, selection is only expected to produce small reductions in phenotypic and genetic variances due only to the generation of linkage disequilibrium (BULMER 1971, 1980). If, however, a small number of genes at substantial frequencies make a relatively large contribution to the genetic variance, larger changes are expected (SORENSEN and HILL 1982). In this situation, changes in genetic variation are brought about by gene-frequency changes in addition to deviations from linkage equilibrium caused by selection. In our case, in which only males are selected, total genetic variation is also affected by the deviations from Hardy-Weinberg produced in the offspring generation.

Let us assume that the difference between the additive genetic components we observed is indeed real, which would be a remarkable result because we are looking at the effect of only one round of selection of moderate intensity ($i = 0.36$). To determine whether this is plausible under various genetic models involving relatively few genes with additive effects, some simulations were performed. The first model, called BULMER (BULMER 1976), involved 12 loci with two alleles each, + and −, at frequencies $p = q = 0.5$, contributing 0 and 1 to the quantitative trait. The genotypic value of an individual is the number of + alleles, and the phenotypic value was obtained by adding a $N(0, 36)$ environmental component (*i.e.*, we assume $h^2 \approx 0.14$). The 12 loci were divided into three groups of four loci each, and each group was assumed to be on a different chromosome [the karyotype of *D. buzzatii* consists of four pairs of equal length acrocentric autosomes, one pair of dot chromosomes, a long acrocentric *X* and a small acrocentric *Y* chromosome (WASSERMAN 1962)]. Nonhomologous chromosomes were assumed to segregate independently, and the recombination fraction between loci on the same chromosome was 0 in males. The genotypes of 200 males were generated at random. To simulate the P males, the 200 males were arranged in rank order by their phenotypic values and the 20% with the lowest ranks were discarded ($i = 0.35$). Eighty randomly selected males were mated at random with 80 females from the base population and each mating pair produced one offspring. To simulate the S males, the 80 parental males were taken at random from the original population of 200 males. Each run was replicated 200 times. The second model, called S&H (SO-

RENSEN and HILL 1982), was similar to BULMER, but one diallelic locus of large effect on the trait was assumed to be located on a different chromosome. This locus accounted for ~5% of the total phenotypic variance [*i.e.*, higher than the 2% value accounted for by the second-chromosome karyotypes in wild flies, where chromosome frequencies are 0.4102 for *2st* and 0.5898 for *2j*· (see RUIZ *et al.* 1991)]. Initial gene frequencies (♦) at this locus were 0.4, 0.5 and 0.6, and h^2 was set to ~0.12. As above, each run was replicated 200 times. The numerical results of these simulations can be summarized as follows. The lowest (BULMER model) and the highest (S&H model with ♦ = 0.6) ratio between the average genotypic variances in the offspring of S males relative to that of P males were ~1.02 and 1.04, respectively. These figures are substantially lower than the 1.57 ratio we observe (Table 3). Therefore, it is difficult to accept that the reduction in additive genetic variance in Table 3 is brought about by directional selection on body size. It must be stressed, however, that while the difference in variance between the two progeny samples provides evidence that there are genetic differences between the paternal samples, it does not provide evidence that selection is acting in a manner that would produce a directional response in the field. It is quite possible that, due to phenotypic selection on other, unmeasured characters genetically correlated with body size, the net genetic selection during the mating stage is in fact stabilizing.

A somewhat surprising result is the significant difference in average development time between the progeny of the S and P males. The difference in mean development time between our samples is small (~5 hr) in comparison with the generation time of the flies. However, it must be remembered that these flies were reared in near optimal conditions. This small difference may in fact represent a much larger difference that may have substantial effects on fitness in natural populations if there is strong larval competition (SANTOS *et al.* 1994). It would appear therefore that in our samples there is a positive correlation between mating success and at least one other fitness component as would be predicted by "good genes" models of sexual selection (MAYNARD SMITH 1991). There is conflicting evidence of the effect of mate choice on offspring fitness in Drosophila. PARTRIDGE (1980) and TAYLOR *et al.* (1987), report positive effects while BOAKE (1986) and SCHAEFFER *et al.* (1984) found no effect. It must be stressed however that neither our data nor those of TAYLOR *et al.* and PARTRIDGE indicate whether there is female choice taking place. In fact there is almost no evidence for female mating preference either in *D. melanogaster* or *D. buzzatii*. Larger males probably owe part of their mating advantage to higher levels of courtship (PARTRIDGE *et al.* 1987a,b; SANTOS *et al.* 1992b). The results may be explained equally well by assuming that the mating males carry a lower than average frequency of generally deleterious mutations that affect body size, mating success and development time. Some evidence that this may be the case comes from the observation of a negative correlation between size and development time in the unmated S males but no significant correlation in the P males. Additionally, the actual difference between the progeny samples in average wing length might not be due to putative directional selection acting on body size in the wild, but on deleterious alleles with pleiotropic side effects.

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