The evolutionary history of Drosophila buzzatii. XX.
Positive phenotypic covariance between field adult fitness components and body size

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Key words: Drosophila; thorax length; age-structure; adult fitness components; natural population.

Abstract

In the cactophilic species Drosophila buzzatii, it is feasible to infer the action of natural selection by simultaneously sampling different life history stages in the field. During four years of research, samples of mating and non-mating adults and pupae were taken from a natural population. The main adult fitness components, i.e., mating success, longevity, and fecundity, were recorded in relation to body size, as measured by thorax length. The age of flies was estimated by observing the developmental stage of the reproductive system. Our data showed that larger flies can outlive and outmate small flies, and that mating success is related to age. An estimate of the fitness function showed a linear increase of mating success with increasing thorax length. There was no assortative mating for this trait. We advance the hypothesis that mating success is related to the rate of encounter and courtship time through general activity, which in turn may be related to body size. A positive phenotypic correlation between thorax length and ovariole number, which is related to fecundity, was found in females emerged from wild pupae. Neither the phenotypic nor the genetic (additive) correlations between these two traits were statistically different from zero in laboratory reared females. The genetic consequences of the observed phenotypic selection on body size are discussed.

Introduction

There is a simple relationship between fitness and phenotypic variation for a given character: the change in the mean value of a character within a generation,
i.e., the selection differential, equals the covariance between relative fitness and character (Robertson, 1966; Price, 1970; Bulmer, 1980; Lande and Arnold, 1983). Natural selection, however, does not occur for single traits in isolation. Phenotypic selection of (sensu Sober, 1984) the studied trait through correlated changes with other characters may be observed. This is the fundamental limitation of univariate measures of selection. A statistical solution to this problem is to measure as many characters as possible and to work out the partial regression coefficients of relative fitness on each character (Lande, 1979; Lande and Arnold, 1983; Arnold and Wade, 1984; Phillips and Arnold, 1989). These coefficients, called the selection gradients, should measure the direct effect of selection on a character. In practice, however, there are a number of complications in the use of the linear regression approach (see e.g. Wetherill, 1986; Mitchell-Olds and Shaw, 1987). A biologically meaningful alternative is to study a well defined, physiologically and ecologically relevant trait. This strategy seeks not only to measure selection but, at the same time, to get some insight into its biological causes (Endler, 1980, 1986).

In Drosophila, as in many other organisms, body size is a universal attribute that is easily measured. It is closely linked to life history traits (Roff, 1981, 1986) and has been widely used in studies on quantitative genetics (e.g., Robertson, 1955). Furthermore, if we are able to correlate detectable genetic variation with body size and enough is known about the ecology of the species, a promising avenue to understand the complex interactions among genotype, phenotype, and ecological variation, is opened (Ruiz and Santos, 1989; Ruiz et al., 1991).

A precise definition of fitness is complex in organisms with overlapping generations like Drosophila. Lifetime reproductive success is surely the best measure of fitness we can have because phenotypic trade-offs may even reverse trends found during a particular selective episode (see Clutton-Brock, 1988). Gathering data for longitudinal studies or cohort analysis (Endler, 1986) seems impossible in nature, but it is feasible in D. buzzatii to obtain simultaneous samples of different life history stages (Barker, 1977; Ruiz et al., 1986). In a previous paper (Santos et al., 1988), we were able to show a short-term mating success associated with larger body size. Copulating and solitary individuals were sampled at particular points in time and measured for thorax length. The relative strengths of natural and sexual selection could not be determined, but this cross-sectional approach provided a good starting point to study the relationship between body size and adult fitness components. Mating (copulatory) success is likely to be related to reproductive success and, in turn, to fitness.

We report here additional data of thorax length frequency distributions in simultaneous samples of wild mating and non-mating D. buzzatii flies. Body size was correlated with relative age of flies, which was estimated by means of the somewhat rough but reliable procedure of observing the developmental stage of the reproductive system. We also present data on the phenotypic and genetic correlations between thorax length and ovariole number. These data were used to relate variation in body size with the main adult fitness components, i.e., mating success, longevity and fecundity. In particular, we wished to assess whether (1) larger flies have higher mating success than smaller ones, as the previous data suggested, or (2)
the variance in adult survivorship is related to body size and this accounts for the apparent mating advantage of larger flies. We conclude that thorax length is positively correlated with those three fitness components and selection for body size is essentially directional in nature.

**Materials and methods**

**Field studies**

A comprehensive account of the ecology and mating behaviour of *D. buzzatii* and full details about the study site and sampling procedures are given in earlier publications (Ruiz et al., 1986; Santos et al., 1988, 1989). No rotting *Opuntia ficus-indica* fruits were present in the sampling area at the time of collections, so *D. buzzatii* flies were feeding and breeding exclusively on rotting cladodes. A total of 5651 wild flies measured for thorax length and grouped according to sex, year and sample, constitute the basic data set used in this paper (Table 1). All measurements were made on live individuals to the nearest 1/40 mm (1987 and 1988) or 1/75 mm (1989 and 1990). The body size data obtained in June 1987 have been previously published (Santos et al., 1988) and are given here for the sake of completeness.

In 1988 and 1990, the reproductive system of each mating and non-mating fly was observed with a high-powered binocular after dissecting the etherized adults placing them in a drop of saline solution on a clean slide. The dissection is made by means of a pair of fine needles, placing one through the thorax and the other through the posterior tip of the abdomen and pulling (Patterson, 1947). Several successive developmental stages in the reproductive systems of males and females from the time of eclosion up to maturation can be easily distinguished (see below).

In 1989, non-mating males were individually placed in vials (2.5 x 8 cm, with a 5 cc of standard cornmeal-agar-yeast food) immediately after capture with two receptive virgin females. After three hours, these males were changed to another vial and the females were allowed to lay eggs. This was done to distinguish between sexually mature and immature males at the time of collection. The bait-trapped males were dissected as above to ascertain the developmental stage of the reproductive system.

Wild pupae from 19 rotting *O. ficus-indica* cladodes, randomly sampled all over the collecting area in 1988, were taken by opening each rot with a scalpel and spreading the rotting plant tissues. The preferred pupation sites of *D. buzzatii* larvae are the cylinders of the dense and highly reticulate vascular system and the inner wall of the epidermis, close to an open hole. Pupae are not always easily visible for their tan colour is very similar to that of the plant tissues. Bunches of pupae were introduced into vials with fresh food and eclosing flies were separated by sex. A total of 126 females from these pupae were measured for thorax length and dissected as above to determine ovariole number.
Table 1. Means of thorax length (in mm) and measures of skewness and kurtosis of all samples of wild *Drosophila buzzatii* flies referred to, or used in, this study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>Mean ± SE (G,)</th>
<th>Skewness (G,)</th>
<th>Kurtosis (G,)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MALES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 1987</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating</td>
<td>352</td>
<td>0.984 ± 0.003</td>
<td>-0.371 ± 0.130</td>
<td>0.385 ± 0.259</td>
</tr>
<tr>
<td>Non-mating</td>
<td>299</td>
<td>0.963 ± 0.004</td>
<td>-0.600 ± 0.141</td>
<td>0.400 ± 0.281</td>
</tr>
<tr>
<td>Bait-trapped</td>
<td>218</td>
<td>0.970 ± 0.004</td>
<td>-1.012 ± 0.165</td>
<td>1.638 ± 0.320</td>
</tr>
<tr>
<td>June 1988</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating</td>
<td>298</td>
<td>1.000 ± 0.004</td>
<td>-0.584 ± 0.141</td>
<td>0.459 ± 0.281</td>
</tr>
<tr>
<td>Non-mating</td>
<td>303</td>
<td>0.983 ± 0.004</td>
<td>-0.296 ± 0.140</td>
<td>0.169 ± 0.279</td>
</tr>
<tr>
<td>June 1989</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating</td>
<td>340</td>
<td>1.017 ± 0.003</td>
<td>-0.953 ± 0.132</td>
<td>1.391 ± 0.264</td>
</tr>
<tr>
<td>Non-mating</td>
<td>397</td>
<td>1.015 ± 0.004</td>
<td>-0.934 ± 0.122</td>
<td>1.093 ± 0.244</td>
</tr>
<tr>
<td>Bait-trapped</td>
<td>352</td>
<td>1.012 ± 0.004</td>
<td>-0.503 ± 0.130</td>
<td>0.446 ± 0.259</td>
</tr>
<tr>
<td>November 1990</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating</td>
<td>83</td>
<td>1.014 ± 0.007</td>
<td>0.642 ± 0.264</td>
<td>0.594 ± 0.523</td>
</tr>
<tr>
<td>Non-mating</td>
<td>213</td>
<td>0.993 ± 0.005</td>
<td>-0.905 ± 0.167</td>
<td>0.943 ± 0.332</td>
</tr>
<tr>
<td><strong>FEMALES</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 1987</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating</td>
<td>321</td>
<td>1.048 ± 0.003</td>
<td>-0.409 ± 0.136</td>
<td>0.340 ± 0.271</td>
</tr>
<tr>
<td>Non-mating</td>
<td>287</td>
<td>1.037 ± 0.004</td>
<td>-0.395 ± 0.144</td>
<td>-0.123 ± 0.287</td>
</tr>
<tr>
<td>Bait-trapped</td>
<td>267</td>
<td>1.031 ± 0.004</td>
<td>-0.349 ± 0.149</td>
<td>0.016 ± 0.297</td>
</tr>
<tr>
<td>June 1988</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating</td>
<td>298</td>
<td>1.064 ± 0.003</td>
<td>-0.397 ± 0.141</td>
<td>0.097 ± 0.281</td>
</tr>
<tr>
<td>Non-mating</td>
<td>300</td>
<td>1.049 ± 0.004</td>
<td>-0.469 ± 0.141</td>
<td>0.164 ± 0.281</td>
</tr>
<tr>
<td>From wild pupae</td>
<td>126</td>
<td>1.044 ± 0.006</td>
<td>-0.527 ± 0.216</td>
<td>0.612 ± 0.428</td>
</tr>
<tr>
<td>June 1989</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mating</td>
<td>361</td>
<td>1.091 ± 0.004</td>
<td>-0.518 ± 0.128</td>
<td>0.675 ± 0.256</td>
</tr>
<tr>
<td>Non-mating</td>
<td>281</td>
<td>1.092 ± 0.004</td>
<td>-0.655 ± 0.145</td>
<td>0.821 ± 0.290</td>
</tr>
<tr>
<td>Bait-trapped</td>
<td>296</td>
<td>1.090 ± 0.004</td>
<td>-0.343 ± 0.142</td>
<td>0.218 ± 0.282</td>
</tr>
<tr>
<td>November 1990</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating</td>
<td>83</td>
<td>1.092 ± 0.008</td>
<td>-0.582 ± 0.264</td>
<td>0.727 ± 0.523</td>
</tr>
<tr>
<td>Non-mating</td>
<td>176</td>
<td>1.074 ± 0.007</td>
<td>-0.767 ± 0.183</td>
<td>0.812 ± 0.364</td>
</tr>
</tbody>
</table>

**Laboratory studies**

**Testes and ovarian development**

A brief account of the male internal reproductive structures is given in Patterson and Wheeler (1942) and Throckmorton (1962a). Previous to the field studies, Dr. Emilio Valadé carried out a test using a *D. buzzatii* strain derived from impregnated females collected in 1977 at Carboneras to correlate reproductive stage with age.
Natural selection on body size in Drosophila (unpublished results). Males and females were raised under near optimal conditions and kept in bottles at 25°C. After hundreds of dissections at different time intervals, up to five reproductive stages in males and up to six in females were evident and easily perceivable for routine work with a large sample of flies.

At eclosion the testes are colourless or nearly so. Pigment cells accumulate drosopetins at relatively high levels as the male ages (Throckmorton, 1962b), so the testes start having a bright yellow colour which turns to dark red when males reach sexual maturity. Results obtained by Dr. Valadé can be summarized as follows. Stage 1 (0–24 hours after eclosion): testes colourless. A thin layer of muscle fibres surrounds each testis. Stage 2 (24–60 hours): testes translucent turning to bright yellow. The seminal vesicle is small. Stage 3 (60–132 hours): testes reddish and prominent. Seminal vesicle well developed. Stage 4 (5–20 days): testes are dark red and very prominent. The walls of the testes appear expanded. A light puncture with the dissecting needle easily breaks the testes and a red fluid with mature spermatozoa spills out. The seminal vesicle is fully developed. Stage 5 (old males): testes almost empty with a disorganized shape. Their surface has a rough appearance and they are very fragile.

The adult female reproductive system is more or less typical for all Drosophila species. It consists of a pair of ovaries, the genital ducts with their accessory structures (spermathecae, seminal receptacle and accessory glands) and the vagina. Each mature ovary is composed of a variable number of parallel ovarioles. A camera lucida drawing of the female reproductive tract of D. buzzatii can be seen in Patterson (1947) and Patterson and Stone (1952). The ovaries of newly eclosed flies are immature and the most advanced egg chambers are in previtellogenic stages. The ovaries quickly expand and reach maturity. Stage 1 (0–8 hours after eclosion): undeveloped ovaries. The ovaries are relatively small, with numerous tracheal branches clearly observed around them. The yolk formation has not commenced in the egg chambers. Stage 2 (8–36 hours): appearance of the first growing oocytes in the most advanced egg chambers. Ovarioles can be identified. Stage 3 (36–60 hours): growing oocytes transforming into mature eggs with the four filaments easily visible. Only the first chamber of each ovariole contains an egg, which is presumably mature at the end of the stage. The following previtellogenic chambers contain growing oocytes. An almost total synchronization was observed in the rate of ovariole development for each ovary. Stage 4 (60–96 hours): a second stratum of mature eggs appears. Growing oocytes in the following previtellogenic chambers. Stage 5 (more than 96 hours): ovaries expanded, filling up of mature eggs. The body cavity of the adult female is almost occupied by the reproductive organs. Ovarioles are easily identified from the basal to the apical part of the ovaries at the onset of the stage. Identification is more tedious in older flies full of ripe eggs which fall apart easily. Stage 6 (old females): ovaries disorganized in a thread-like fashion, without mature eggs nor oocytes. Ovarioles unidentifiable. In these observations, there was no direct process from undeveloped (stage 1) to postmature (stage 6) without passing the fully mature (stage 5) period. Because of this, it can be said that the postmature ovary is the degenerated one.
Sexual dimorphism for age at reproductive maturity has been described for *D. buzzatii*, with females reaching maturity earlier than males (Barker and Fredline, 1985). Time to male copulation and to female sexual receptivity (i.e., the time at which the female will first accept the male) was estimated in the laboratory (25° C). It is correlated with the maturation of testes and ovaries. Both males and females are able to copulate from reproductive stage 3 onwards, although a few individuals may copulate at stage 2. The question that naturally arises is whether or not the different stages of male and female reproductive systems described above are distinguishable in wild flies. After dissecting 829 males and 1191 females collected at Carboneras before the field experiments, there was no doubt that all previously described reproductive stages could be recognized, although identification is more tedious in starved individuals. At the moment, there is no way to know whether the timing observed in the laboratory is also strictly valid in the field, so any mention to absolute age (in days) is to be taken with caution. It does seem justified to assess the relative age of the flies, this being measured by the developmental stages of the reproductive systems. It is important to point out that the existence of unfavourable conditions for adult females could result in a faster ovary degeneration in wild females than in laboratory reared ones (Watabe and Beppu, 1977). If this were also true for males, our age estimates based on the laboratory timing would be “upper limits”.

Additive genetic correlation and heritabilities of thorax length and ovariole number

Narrow sense heritabilities and their standard errors were estimated from offspring-parent regression (see Ruiz et al., 1991, for experimental details). Genetic (additive) correlation was calculated following Becker (1984 p. 134) as:

\[
\rho_A = \frac{1/2(\text{cov} \ xy' + \text{cov} \ x'y)}{\sqrt{\text{cov} \ xx'}(\text{cov} \ yy')}
\]

\[
\text{S.E.}(\rho_A) = \frac{1 - \rho_A^2}{\sqrt{2}} \times \sqrt{\frac{\text{S.E.}(h_i^2) \times \text{S.E.}(h_j^2)}{h_i^2 \times h_j^2}}
\]

where \(x\) and \(y\) are the values of thorax length and ovariole number in mothers \((n = 262)\), and \(x'\) and \(y'\) are the averages of those values in the two daughters.

Weather records

Daily minimum, average, and maximum temperature data for the months May–June (1987–1989) and October–November (1990) were obtained from the nearest meteorological station (Carboneras, 2–3 km south-west from the collecting site). The recorded average values for every month, together with the absolute minimum and maximum values (in parentheses) to appreciate the range of temperatures, were as follows. May 1987: 19.1° C (11.9°–27.5°). June 1987: 22.7° (15.7°–31.4°). May 1988: 19.3° (12.8°–27.0°). June 1988: 22.3° (16.0°–28.6°). May 1989:
19.1° (10.7°–26.1°). June 1989: 23.4° (16.5°–34.5°). October 1990: 20.4° (13.0°–27.1°). November 1990 (days 1–3): 16.4° (11.5°–20.7°). The per week change in average temperature from May to June was about 0.8° in 1987, 0.7° in 1988, and 1.0° in 1989; and about −1.1° from October to November in 1990.

**Statistical analyses**

Thorax length distributions tended to be leptokurtic and skewed in the direction of smaller size (Table 1). Throughout the paper we test for statistical significance by using robust, conventional parametric statistics. However, non-parametric statistics (e.g., the sign test, the Kruskall–Wallis test, the Wilcoxon test, and the Spearman rank-correlation coefficient) were also carried out and in all cases the same conclusions were reached. Complete descriptions of the standard statistical tests employed can be found in Steel and Torrie (1980) and Sokal and Rohlf (1981).

Multiple regression analyses for the variables thorax length (dependent), age and sample (independent) were performed in 1988 and 1990 samples. The general linear model utilized was:

\[ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \epsilon. \]

where \( Y \) = mean thorax length (in mm), \( X_1 \) = age (measured as developmental stage of the reproductive system), \( X_2 \) = sample (non-mating = 0, mating = 1), and \( X_1 X_2 \) = age by sample interaction (see Kleinbaum et al., 1988, pp 262–276). Given that explanatory variables could be regarded as approximately normal, the F-test for multiple regression is insensitive to non-normality of thorax length (Wetherill, 1986). This multiple regression model yields the following two linear models for the two values of \( X_2 \):

\[ X_2 = 0: \quad Y_N = \text{mean thorax length of non-mating flies} = \beta_0 + \beta_1 X_1 + \epsilon. \]

\[ X_2 = 1: \quad Y_M = \text{mean thorax length of mating flies} = (\beta_0 + \beta_2) + (\beta_1 + \beta_3) X_1 + \epsilon. \]

The interpretation of the coefficients (\( \beta \)) is as follows:

- \( \beta_0 \) = expected mean thorax length for newly emerging wild flies (age 0).
- \( \beta_1 \) = effect of age on mean thorax length.
- \( \beta_2 \) = differential effect of mating on mean thorax length.
- \( \beta_3 \) = differential effect of mating on the relationship between age and thorax length.

It should be noted that the analysis of covariance to compare age-adjusted means is the particular case when \( \beta_3 \) does not differ statistically from 0. Data manipulation and analyses were accomplished on a Vax-8800 VMS at the Centro de Cálculo de la Universidad Autònoma de Barcelona. Statistical programs utilized were those of the BIOM package (Rohlf, 1982) and part of the BMDP Statistical Software (1988).
Results

Body size correlates with mating probability

During the four years, a total of 1163 mating pairs, 1278 non-mating females and 1306 non-mating males were collected. Evidence for a positive relationship between body size and mating probability is available from counting the number of independent collections where mating flies were larger than non-mating ones in those cases where a comparison is possible. These data are chronologically presented in Table 2 and the evidence that larger flies mate more often in the natural population is overwhelming for males and suggestive for females. Also, the variances of thorax length among the mating flies were consistently smaller than those of the non-mating flies. When all the independent collections are considered (Table 2), the two-tailed sign test was statistically significant for both males and females. This shows that mating flies are a selected subset of the entire population.

The standardized selection differentials and the correlations in size between the sexes in the mating pairs are shown in Table 3. The selection intensities were calculated from the standardized difference between the thorax length means of mating and non-mating flies, and statistical significance was assessed by t-tests (Endler, 1986). Although single flies were usually more active and difficult to catch using an aspirator than copulating pairs, we have previously argued that no bias in the estimation of the average thorax length of non-mating flies due to the collecting method exists (Santos et al., 1988). This was corroborated again in the sample of June 1989 where mouth-suctioned and bait-trapped individuals gave similar mean values (t = 0.57, P = 0.569; and t = 0.32, P = 0.749; for males and females, respectively).

Table 2. Two-tailed sign tests for mean thorax length and standard deviation comparisons between subsamples of wild mating and non-mating Drosophila buzzati adults caught in four successive years in the population of Carboneras (Spain).

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of independent collections</th>
<th>Mating flies</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>larger</td>
<td>P</td>
</tr>
<tr>
<td>June 1987</td>
<td>24</td>
<td>20</td>
<td>0.002</td>
<td>16</td>
</tr>
<tr>
<td>June 1988</td>
<td>26</td>
<td>21</td>
<td>0.003</td>
<td>15</td>
</tr>
<tr>
<td>June 1989</td>
<td>21</td>
<td>19</td>
<td>0.000</td>
<td>14</td>
</tr>
<tr>
<td>November 1989</td>
<td>12</td>
<td>7</td>
<td>0.774</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>67</td>
<td>0.000</td>
<td>54</td>
</tr>
<tr>
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<td></td>
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<td></td>
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<tr>
<td>MALES</td>
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<tr>
<td>FEMALES</td>
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</tr>
<tr>
<td>June 1987</td>
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<td>0.152</td>
<td>19</td>
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<tr>
<td>June 1988</td>
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<td>20</td>
<td>0.002</td>
<td>14</td>
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<td>June 1989</td>
<td>21</td>
<td>8</td>
<td>0.383</td>
<td>9</td>
</tr>
<tr>
<td>November 1990</td>
<td>13</td>
<td>10</td>
<td>0.092</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>54</td>
<td>0.005</td>
<td>51</td>
</tr>
</tbody>
</table>
Table 3. Standardized selection differentials ($i$) for body size of Drosophila buzzatii adults caught in four successive years in the population of Carboneras (Spain). Pearson correlation coefficients for thorax lengths of male and female mating pairs are also given for each year (sample sizes for correlations are, respectively, 284, 298, 320 and 83).

<table>
<thead>
<tr>
<th>Date</th>
<th>Sex</th>
<th>$i$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 1987</td>
<td>Males</td>
<td>0.34***</td>
<td>-0.050 ns</td>
</tr>
<tr>
<td></td>
<td>Females†</td>
<td>0.16*</td>
<td></td>
</tr>
<tr>
<td>June 1988</td>
<td>Males</td>
<td>0.25**</td>
<td>0.023 ns</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.24**</td>
<td></td>
</tr>
<tr>
<td>June 1989</td>
<td>Males</td>
<td>0.30***</td>
<td>-0.013 ns</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>-0.01 ns</td>
<td></td>
</tr>
<tr>
<td>November 1990</td>
<td>Males</td>
<td>0.27*</td>
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</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.20 ns</td>
<td></td>
</tr>
</tbody>
</table>

† A modified $t$-test ($t'$; Cochran and Cox [1957, p. 100], Sokal and Rohlf [1981, p. 411]) was applied due to the significantly higher variance of the character in the non-mating females (see Table 2). $n$ $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Six out of eight selection differentials are statistically significant; selection being stronger in males than in females ($\bar{i} = 0.29$ for males and $\bar{i} = 0.15$ for females). An exception to the overall pattern of directional selection on body size was observed in June 1989 and November 1990 when copulating and solitary females did not differ in mean thorax length. Correlation coefficients for thorax length of male and female mating pairs were not statistically different from zero, pointing out that no assortative mating occurs for body size in D. buzzatii.

Body size correlates with age

Table 4 shows the estimated standard partial regression coefficients for the general linear model (see Material and Methods). Given that repeated observations of thorax length for a given age class were obtained, we tested $\text{MS}_{\text{Deviations}}$ over $\text{MS}_{\text{Residual}}$ to check for the presence of linear regression in each sample (Sokal and Rohlf, 1981, pp. 477–491). No significant departures from linear regression were detected in any case. The backward strategy depicted in Kleinbaum et al. (1988 p. 275) was used to test for significance of the partial regression coefficients. In all cases, older individuals were larger than younger ones ($\beta_1$ coefficients). In addition, it seems that some differential effect of mating on the relationship between thorax length and age exists ($\beta_1$ coefficients). In non-mating flies the age-thorax length correlations were statistically significant for both males ($r = 0.249$, $P = 0.000$, in 1988; and $r = 0.138$, $P = 0.047$, in 1990) and females ($r = 0.444$, $P = 0.000$, in 1988; and $r = 0.162$, $P = 0.039$, in 1990); whereas in mating flies the correlations were statistically significant for females ($r = 0.165$, $P = 0.005$, in 1988; and $r = 0.275$,}
P = 0.016, in 1990) but not for males (r = 0.069, P = 0.235, in 1988; and
r = −0.048, P = 0.668, in 1990). Thus, a highly coherent pattern is observed in
both years. In the males bait-trapped in June 1989, a comparable result with that
obtained in June 1988 for non-mating males was observed (r = 0.336, P = 0.000).
Variation in lifespan due to differences in body size could explain the positive
relationship between thorax length and age, although some environmental effects
cannot be discarded (see Discussion).

Strengths of natural and sexual selection on body size

Turning back to Table 3, it is now obvious that the age-structure of natural
populations of D. buzzatii produces a source of variation in thorax length which
must be taken into account when comparing the size of mating and non-mating
flies. The proportion of fully mature flies (age classes 3–4 in males, and 3–5 in
females) was, as expected, significantly higher in mating than in non-mating
individuals (95% vs 78% in 1988 and 99% vs 73% in 1990 for males; 82% vs 59% in
1988 and 84% vs 36% in 1990 for females). Incidentally, the distribution of
mating individuals among the age classes was in accordance with what should be
expected from the laboratory observations (see Material and Methods). Thus, time
to sexual maturity was lower in females (about 20% belonged to age classes lower
than 4, i.e., were younger than 60 hours as estimated in the laboratory at 25° C)
than in males (about 2% were younger than 60 hours).

To test whether or not body size is really related to mating success, mean thorax
length should be adjusted for the difference between mating and non-mating flies in
the covariate age (see Material and Methods). It is convenient to deal first with the
female’s data. The general linear model obviously accounts for a significant amount
of variation in mean female thorax length (R^2 = 0.135, P = 0.000 in 1988; and
R^2 = 0.044, P = 0.015 in 1990), and shows that the two straight-line regression
equations (Table 4) have unequal intercepts and slopes in 1988. Dealing now with
the thorax lengths of males, the analysis did not detect statistically significant
differences between the two straight lines in any year, although no linear regression

Table 4. Standard partial regression coefficients obtained from the general linear model to describe the
relationship between thorax length (dependent variable), and age, sample (non-mating and mating) and
age by sample interaction (independent variables). See text for details.

<table>
<thead>
<tr>
<th>Standard coefficient</th>
<th>June 1988</th>
<th>November 1990</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>β'₁</td>
<td>0.2264***</td>
<td>0.4131***</td>
</tr>
<tr>
<td>β'₂</td>
<td>0.4197*</td>
<td>0.3861**</td>
</tr>
<tr>
<td>β'₃</td>
<td>−0.3651</td>
<td>−0.3681*</td>
</tr>
</tbody>
</table>

* 0.10 > P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001.
Table 5. Standardized sexual selection differentials ($i_s$) for body size of wild mating and sexually mature non-mating Drosophila buzzatii flies (see text for details).

<table>
<thead>
<tr>
<th>Date</th>
<th>Non-mating</th>
<th>Mating</th>
<th>$i_s$</th>
<th>% total $i$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 1988</td>
<td>0.992</td>
<td>0.999</td>
<td>0.10</td>
<td>40</td>
</tr>
<tr>
<td>SD</td>
<td>0.068</td>
<td>0.061</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>230</td>
<td>281</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 1989</td>
<td>1.024</td>
<td>1.037</td>
<td>0.20**</td>
<td>67</td>
</tr>
<tr>
<td>SD</td>
<td>0.068</td>
<td>0.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>312</td>
<td>340</td>
<td></td>
<td></td>
</tr>
<tr>
<td>November 1990</td>
<td>0.999</td>
<td>1.014</td>
<td>0.21</td>
<td>78</td>
</tr>
<tr>
<td>SD</td>
<td>0.073</td>
<td>0.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>151</td>
<td>82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEMALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 1988</td>
<td>1.052</td>
<td>1.064</td>
<td>0.19*</td>
<td>79</td>
</tr>
<tr>
<td>SD</td>
<td>0.062</td>
<td>0.060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>753</td>
<td>798</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$ns P > 0.05; *P < 0.05; ** P < 0.01.$

was found for mating males whereas there was a positive and significant regression for non-mating ones (see above). Table 4 shows the standard partial regression coefficients of both sexes for comparison. It is evident that an overall qualitatively similar situation is observed (Fig. 1).

The difference in mean thorax length between mating and non-mating males within each age class were: $0.20 (t = 2.35, P = 0.020)$ in 1988 and $0.021 (t = 1.60, P = 0.112)$ in 1990 for age class 3; and $-0.004 (t = 0.29, P = 0.775)$ in 1988 and $0.007 (t = 0.46, P = 0.649)$ in 1990 for age class 4. It seems that mating success in relation to body size is age-specific in the natural population. A further insight can be obtained from the analysis of the June 1989 samples when non-mating males were immediately placed after capture with two receptive virgin females during three hours, which allows a direct comparison of thorax length between mating males and sexually mature non-mating males (about 79% of these males gave offspring in the first vial).

Table 5 shows the standardized selection differentials attributed to sexual selection ($i_s$) on body size, i.e., the values obtained by comparing the mean thorax length of actually mated males with that of sexually mature but solitary males. If mating success in relation to body size is age-specific as suggested above, $i_s$ values should be analyzed taking into account the age-structure of the population. Males in 1988 and 1990 samples did not differ in mean relative age (Wilcoxon $z = 1.32, P = 0.187$), but were younger than those in 1988 ($z = 4.22, P = 0.000$; and $z = 4.78, P = 0.000$, respectively). Hence, the fact that $i_s$ values were comparable in 1989 and 1990, but both were higher than the value obtained in 1988, confirms that $i_s$ is dependent on the age-structure of the population, i.e., the younger the population,
the higher the difference in mean thorax length between mating and non-mating flies. Taking all years together, roughly 60% of the directional selection on body size for males in Table 3 can be attributed to sexual selection. For the female samples in June 1988 the figure is about 79%, although the \( i_s \) value obtained may be an overestimate as females from age class 2 up to 6 were considered and non-mating females from age class 2 could be immature.

**Fitness functions in relation to body size**

Since *D. buzzatii* individuals have different probabilities of mating as a function of body size, it is of interest to know the fitness function that relates mating success of flies to the trait thorax length. For this purpose, we used the non-parametric cubic-spline approach described by Schluter (1988). This approach provides a fairly clear demonstration of the type of selection, directional (linear), stabilizing or disruptive (non-linear), that is operating on this particular trait.

The data used to estimate fitness functions were those in Table 5, when the standardized sexual selection intensity was statistically significant. The spline estimate of relative mating success on thorax length showed a positive fairly linear association between mating success and thorax length (Fig. 2). Sexual selection was purely directional, and important differences in fitness accompanying slight to moderate differences in thorax length can be observed.

**Body size and ovariole number: heritabilities and correlations**

The number of ovarioles per ovary varies widely among females, wild and laboratory-raised alike. Nevertheless, we have observed slightly higher (not statisti-
Fig. 2. Non-parametric estimates of sexual selection fitness functions of wild Drosophila buzzatii males (June 1989) and females (June 1988) in relation to thorax length.

cally significant) phenotypic variation for ovariole number in the D. buzzatii females emerged from wild pupae (C.V. = 17.2%) than in those raised in the laboratory (C.V. = 15.9%), and a (significantly) lower number of ovarioles per ovary (ranging from 6 to 27, with an average value of 17) in the former than in the latter (ranging from 7 to 27 with an average value of 17). These observations agree with those made for D. mimica by Kambysellis and Heed (1971). The difference still holds true even when similar sized females are compared.

The phenotypic correlation between thorax length and ovariole number per fly, calculated using the numerical scale for both characters, was positive in the females emerged from the wild pupae ($r_p = 0.345$, $P = 0.000$). It is well known that positive phenotypic correlations between these traits exist, provided there is appreciable variation in Drosophila larval nutrition (Robertson, 1957a).

Laboratory estimates of narrow sense heritability for thorax length (off-spring-midparent regression) and ovariole number (daughter-mother regression) were, respectively, $0.534 \pm 0.040$ (Ruiz et al., 1991) and $0.763 \pm 0.097$. These values are higher than those reported in the literature for other Drosophila species (Mahowald and Kambysellis, 1980; Roff and Mousseau, 1987), but the one for thorax length is comparable to those reported by Robertson (1987) and Prout and Barker (1989) for several Australian populations of D. buzzatii. Neither the phenotypic ($r_p = -0.001 \pm 0.062$) nor the additive ($r_A = -0.097 \pm 0.076$) correlations were statistically different from zero.
Discussion

Phenotypic selection on body size and reproductive biology of D. buzzatii

The data reported in this paper provide evidence for a positive correlation between body size and the three major adult fitness components, namely, longevity, mating success and fecundity, in a natural population of the cactophilic fly D. buzzatii. We shall now discuss the nature of the evidence and will consider later which might be the genetic consequences of the directional selection operating on body size.

In non-mating flies of both sexes a significant positive correlation was observed between thorax length and relative age. This correlation may be interpreted as prima facie evidence for body size related differences in adult survivorship. However, in cross-sectional studies, like those reported here, inferences about selection are drawn from a comparison of phenotypic distributions between cohorts of different age sampled from a population at a single point in time. Two underlying assumptions in these studies are (Lande and Arnold, 1983; Endler, 1986): (1) that there is no ontogenetic change for the studied trait between the life-cycle stages being compared, and (2) that individual development of the character was not affected by the environment. In Drosophila, the size of the adult is fixed at emergence, so assumption (1) is satisfied in our analysis. On the other hand, a number of environmental factors such as temperature (Parsons, 1961; Tantaway and Mallah, 1961; Tantaway, 1964; Robertson, 1987), larval crowding (Sang, 1950; Miller, 1964; Atkinson, 1979b; Grimaldi and Jaenike, 1984), and larval nutrition (Robertson, 1989), are known to affect those phenotypic traits normally used as indices of adult body size (i.e., thorax length and wing length). These factors are not independent, for temperature also has an effect on the population dynamics of Drosophila (Atkinson and Shorrocks, 1977).

As in most Drosophila species, thorax length in D. buzzatii decreases with increasing temperature (Robertson, 1987). Hence, larger adults are expected to emerge earlier in the year in the summer samples, whereas the opposite is expected in fall. Under the assumption that mean air temperature was the main environmental factor in the among-cohorts variation for body size, a positive relation between age and thorax length is expected in June and a negative one is expected in November. Test of homogeneity for the three correlation coefficients (Sokal and Rohlf, 1981, pp. 583–591) in non-mating and bait-trapped males shows that age-thorax length relationship was not the same among samples ($X^2 = 5.79, P = 0.016$). The difference is due to the lower correlation in November than in June. A similar result was obtained for non-mating females ($t = 3.16, P = 0.002$). Thus, an environmental effect is apparent from the data. However, the fact that thorax length also increases with age in the fall 1990 sample (Table 4) strongly argues for longevity differences due to variance in body size.

The finding that larger flies outlive smaller ones agrees quite well with laboratory studies in other Drosophila species (Tantaway and Vetukhiv, 1960; Partridge and Farquhar, 1983). This result might have been predicted from the abundant litera-
ture on the effects of animal body size on specific metabolic rates, starvation, thermal equilibration, locomotion, and predation (Peters, 1983; Calder, 1984). In *Drosophila*, for instance, larger flies have been shown to have a relatively lower standard metabolic rate (Egges and Klassen, 1989), a higher desiccation tolerance (Levins, 1969; Parsons, 1970) and a greater dispersal ability (Roff, 1977). All these features are probably quite relevant under natural conditions.

Mating success in relation to body size was age-dependent and the data suggest that larger flies outmated smaller ones when these were relatively frequent, i.e., earlier in life. When all the information from the four years is considered jointly, it is clear that the difference in size between mating and non-mating flies is greater and more consistent through time in males than in females (Tables 2 and 3), a result in good agreement with field observations made in other *Drosophila* species (Partridge et al., 1987; Taylor and Kekić, 1988). Roughly speaking, male mating success accounted for 60% of the total selection intensity detected (cf., Tables 3 and 5) and was linearly related to body size in the field, as it seems to be in other species in the laboratory (Ewing 1961, 1964; Monclus and Prevosti, 1971; Partridge and Farquhar, 1983; Wilkinson, 1987).

Why do larger flies have a higher mating success in nature? From the information we have on the biology of *D. buzzatii*, there is no evidence of male–male aggressive interactions nor mate choice in the field. The mating pattern in the natural population of Carboneras is at random for phenotypic characters such as body size (Table 3) and age (contingency chi-squares for male and female age classes in 1988 and 1990 were not statistically significant), as well as for genetic markers (Quezada-Díaz et al., 1992). As soon as a female approaches a group of males, one or more males start courting. Courtship time was not measured, but differences among males for this variable are easily observed. What seems to us the most convincing explanation is that mating success is related to the rate of encounter and courtship time through a fly’s general activity, and this is related to body size. It would be interesting to carry out field observations in order to correlate body size, period of adult activity, and mating success. This is not an easy task in *Drosophila* for individual recognition is almost impossible. However, this stands as a challenge for future field work.

Male reproductive success is determined by the number of matings achieved, whereas female reproductive potential is determined by the number of eggs laid. It has been questioned whether or not variance in the number of matings achieved by females is relevant for fecundity, but Ridley’s (1988) recent review shows that, in general, repeated mating increases this fitness component in insects. In the laboratory, *D. buzzatii* female remating is relatively frequent, and the number of offspring produced by a female depends on the number of mates (Barbadilla et al., 1991). This is in accordance with Markow’s (1982) studies of female remating in cactophilic *Drosophila*. Under field conditions, where larger differences in yeast diet exist, and females may run out of sperm, variance in the amount of sperm transferred by males may have important consequences on the female’s willingness to remate (see Starmer et al., 1988; Steele, 1986a, b) and egg productivity.
The reproductive strategy of *D. buzzatii* is consistent with that described by Kambysellis and Heed (1971) and Atkinson (1979a) for drosophilids breeding on nutritionally rich, yet relatively infrequent, substrates. *D. buzzatii* possesses numerous synchronously developing ovarioles, each containing more than one mature egg. Successful oviposition events seem to be rare in nature, and the laying female probably deposits a clutch of eggs. This is concluded from field observations on the relatively high number of visits to rotting *O. ficus-indica* cladodes, in contrast with the limited number of parents breeding on each site (Santos et al., 1989). As reported for different *Drosophila* species (Robertson, 1957a, b; Kambysellis and Heed, 1971; Grimaldi and Jaenike, 1984; Heed and Mangan, 1986), a positive phenotypic correlation between ovariole number, which is related to fecundity in wild and laboratory underfed flies, and thorax length was found in *D. buzzatii* females emerged from wild pupae. However, neither the phenotypic nor genetic correlations for well fed, laboratory reared females, were statistically different from zero. Robertson (1957a) has shown that individual variation in ovariole number is very high in genetically uniform flies and is unimportant with respect to egg production, provided the larvae have been reared under optimal conditions of food. As pointed out by Roff (1981 p. 415), “the actual relationship between fecundity and size appears to be mediated most directly through phenotypic size rather than genotype”.

**Genetic consequences of phenotypic selection on body size**

Phenotypic variance for thorax length in wild *D. buzzatii* flies is very high, which is partly, perhaps mostly, due to the heterogeneity of larval habitats in temperature, yeast abundance and yeast diversity (Barker, 1982; Fogelman, 1982; Barker et al., 1983, 1987). In spite of the potentially huge effect of ecological factors, significant additive genetic variance for thorax length has been detected in nature (Prout and Barker, 1989; Ruiz et al., 1991). Hence, the question naturally arises: are the *D. buzzatii* flies in the population of Carboneras increasing in size, or, on the contrary, is the population at (genetic) equilibrium due to compensating disadvantages in growing to a larger size? Theoretical considerations aside, the answer will come from empirical studies of mean thorax length of laboratory progenies obtained from wild collected flies. We have started such analysis by regularly sampling isofemale lines and rearing the F2 flies under environmentally controlled conditions, but the data are still insufficient to obtain meaningful conclusions.

There is appealing indirect evidence for compensating selection against larger size during other phases of the life cycle. Artificial selection for larger body size in *D. melanogaster* may be accompanied by an increase in development time (Robertson, 1960) without change in larval growth rate (Robertson, 1963), and higher larval mortality under some conditions (Reeve and Robertson, 1953; Wilkinson, 1987; Santos and Partridge, in preparation). Additionally, interspecific comparisons show that large bodied *Drosophila* species have slow larval development, whereas small bodied species show the opposite characteristic (Atkinson, 1979a). Mangan (1982)
Natural selection on body size in Drosophila

has observed a positive relationship between female thorax length and cactus size for different Drosophila species of the repleta group breeding on different columnar cacti. This is as expected, for larger cacti produce more persistent rots giving flies longer to develop.

In D. buzzatii, a positive relationship between development time and juvenile mortality might be taking place in nature. We were able to show that there are fitness differences among second-chromosome karyotypes for the egg-to-third-instar larva viability component in the population of D. buzzatii at Carboneras (Ruiz et al., 1986; Santos et al., 1989), and that about 2% of the phenotypic variance for thorax length in wild males is accounted for by the second chromosome inversions (Ruiz et al., 1991). We hope to further analyze the relationships among karyotypes, body size, viability, and development time. For the time being, the information is only conjectural, but the research promises to be fruitful.

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