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ANTAGONISTIC PLEIOTROPIC EFFECT OF SECOND-CHROMOSOME INVERSIONS ON BODY SIZE AND EARLY LIFE-HISTORY TRAITS IN *DROSOPHILA BUZZATII*

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Abstract.—A simple way to think of evolutionary trade-offs is to suppose genetic effects of opposed direction that give rise to antagonistic pleiotropy. Maintenance of additive genetic variability for fitness related characters, in association with negative correlations between these characters, may result. In the cactophilic species *Drosophila buzzatii*, there is evidence that second-chromosome polymorphic inversions affect size-related traits. Because a trade-off between body size and larval developmental time has been reported in *Drosophila*, we study here whether or not these inversions also affect larva-adult viability and developmental time. In particular, we expect that polymorphic inversions make a statistically significant contribution to the genetic correlation between body size (as measured by thorax length) and larval developmental time. This contribution is expected to be in the direction predicted by the trade-off, namely, those flies whose karyotypes cause them to be genetically larger should also have a longer developmental time than flies with other karyotypes. Using two different experimental approaches, a statistically significant contribution of the second-chromosome inversions to the phenotypic variances of body size and developmental time in *D. buzzatii* was found. Further, these inversions make a positive contribution to the total genetic correlation between the traits, as expected by the suggested trade-off. The data do not provide evidence as to whether the genetic correlation is due to antagonistic pleiotropic gene action or to gametic disequilibrium of linked genes that affect one or both traits. The results do suggest, however, a possible explanation for the maintenance of inversion polymorphism in this species.

Key words.—Antagonistic pleiotropy, body size, developmental time, *Drosophila*, genetic correlation, polymorphic inversions, trade-offs, viability.

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Theories about the evolution of life histories generally demand functional constraints on what organisms can achieve in practice. One form that these constraints takes is a trade-off between two or more fitness traits. Trade-offs that give rise to antagonistic pleiotropy can in turn lead to the maintenance of additive genetic variability for fitness-related characters, in association with negative genetic correlations between these characters (Rose 1982; Rose et al. 1987). Genetic correlations are usually estimated by a comparison of variation among families or from correlated responses to selection. Reznick (1985, 1992) reviewed the different methods for assessing life-history trade-offs, and concluded that only estimated genetic correlations provide the essential information necessary to establish the existence of trade-offs. Functional constraints should be, therefore, generally viewed as genetic constraints imposed by pleiotropy.

The evidence for trade-offs in fitness components comes primarily from the observation of correlated responses to selection. For instance, in *Drosophila melanogaster* some studies suggest that adult body size, a fitness-related trait, exhibits a trade-off with larval developmental rate. Thus, artificial selection for large adult body size results in an increase in larval developmental time with a consequent drop in larval viability under high densities (Robertson 1960; Santos et al. 1992a, 1994; Partridge and Fowler 1993). Conversely, selection for fast larval development reduces adult body weight and, hence, lifetime fecundity (Nunney 1996). These results suggest the existence of individual genes exerting antagonistic effects on both traits. The next step would be to focus on candidate loci that cause quantitative variation in, say, body size, and demonstrate that they also exert an effect on developmental time in the direction predicted by the trade-off. There are, however, at least two problems with this approach. First, only in the past few years has it become feasible to develop dense molecular marker maps, and to map quantitative trait loci (QTLs) in some species (see, e.g., Mackay 1995; Mitchell-Olds 1995a). We are still a long way from knowing the genetic details of quantitative variation in most traits. Second, even if antagonistic gene effects are common, theoretical models suggest that the number of loci on which this type of selection can be operating to maintain a genetic polymorphism is probably very small, if at all (Rose 1982; Curtsinger et al. 1994; for an example of a candidate locus, see Charlesworth 1996). In addition, extensive studies in *Drosophila* provide little support for overdominant loci significantly contributing to fitness variance in natural populations (Charlesworth and Charlesworth 1987; Houle 1989; Houle et al. 1996). Therefore, large-scale QTL mapping might be a quite frustrating approximation to test for balancing selection due to antagonistic pleiotropy.

A complementary approach could be to focus on a chromosome fragment (say inversion) known to cause quantitative variation in a fitness trait and study whether this fragment makes a significant contribution to the genetic correlation with another fitness trait. Even though clear evidence for pleiotropic gene action could not be obtained because of the possible gametic disequilibrium of linked genes affecting one

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or both traits, an overdominant locus could be eventually mapped if it exists (see Mitchell-Olds 1995b). Recent studies have shown effects of second-chromosome inversions on adult body size in the cactophilic species *D. buzzatii* (Ruiz and Santos 1989; Ruiz et al. 1991; Hasson et al. 1992; Norry et al. 1995). This chromosome has been shown to be polymorphic for up to six paracentric inversions (Ruiz et al. 1984; Ruiz and Wasserman 1993), but most natural populations are usually polymorphic for the two common cosmopolitan arrangements *2st* and *2j* (Fontdevila et al. 1981, 1982; Barker et al. 1985; Knibb et al. 1987; Hasson et al. 1995). The effect of the karyotype on body size is thought to be caused by the association of different inversions with particular alleles that influence this trait (Ruiz et al. 1991).

Here, we test whether or not these inversions also affect larval developmental time or larval viability. If body size and these fitness traits are genetically coupled, as suggested by the correlational studies cited above, this would be revealed as a statistically significant contribution of the polymorphic inversions to the total genetic correlation, and would provide the necessary basis to a possible explanation for the maintenance of inversion polymorphism in this species.

Materials and Methods

Origin of Flies and Experimental Procedures

Samples of the cactophilic species *D. buzzatii* were collected from two natural populations on the Mediterranean coast of Spain: Carboneras (Almería; 37°N, 1°9′W) and Colera (Girona; 42°4′N, 3°2′E). The population at Carboneras is described in detail in Ruiz et al. (1986), and that at Colera in Betrán et al. (1995). Both populations are polymorphic for the two common cosmopolitan second-chromosome arrangements *2st* and *2j*, as well as for the rare cosmopolitans $2jz^3$ and $2jq^7$ (for a description, see Ruiz et al. 1984). Throughout the experiments CO_2 was used when anesthesia was necessary.

Experiment A.—A large sample of adult flies was caught with banana baits at Carboneras in November 1990. After despermatization of females (see Barbadilla et al. 1991; Ruiz et al. 1991), 278 mating pairs were set up in small vials with 5 mL of food. The salivary gland chromosomes of up to eight offspring larvae from each mating pair were analyzed to infer the parental genotypes (Barbadilla and Naveira 1988). Fifty vials with suitable parental genotypes were kept and the rest discarded. Two inversions (B and C) could be made isogenic from vials with parental genotypes AB × AC or AA × BC (each capital letter stands for a different chromosome arrangement), whereas isogenic strains for any of the four inversions could be obtained from vials with parental genotypes AB × CD. After three to six generations of sib-mating and selection of flies with the desired arrangement, six independent isogenic lines (e.g., A1 . . . A6) were obtained for each of the four second chromosome arrangements (*2st, 2j,* $2jz^3$, and $2jq^7$). The mean expected inbreeding coefficient for the 24 lines (excluding the inversion segment made isogenic) was 0.638.

The experimental flies were four-way hybrids obtained by crossing the 24 inbred strains as follows. First, the six lines with a given arrangement were crossed to produce the three independent two-way hybrids (e.g., A1A2, A3A4, and A5A6 plus reciprocals). Then, homokaryotypic larvae for each arrangement were obtained crossing two-way hybrids with the same arrangement (e.g., A1A2 × A3A4, A1A2 × A5A6, and A3A4 × A5A6 plus reciprocals) and heterokaryotypic larvae in a similar way but using two-way hybrids with different arrangements (e.g., A1A2 × B1B2, A3A4 × B3B4, and A5A6 × B5B6 plus reciprocals). Virgin males and females for each mating combination were placed in a plastic chamber with live yeast for egg collection. Hatching first instar larvae (± 5 h) were placed in vials with fresh culture medium and incubated at 22°C (12:12 L:D cycle) with uncontrolled humidity. Two larval densities were used: 20 larvae in 5 mL of food (low density) and 40 larvae in 2 mL of food (high density).

Each replicate of the experiment consisted of 120 vials: 10 vials for the different karyotypes × three vials for the different strains used to obtain the tetrahybrid first-instar larvae × two vials for the direction of the crosses (maternal effects) × two vials for the two larval densities. The full experiment was replicated five times, so a randomized block design was used.

The total number of pupae in each vial was scored. Emerged adults were collected every 12 h and fixed in 3:1 alcohol:glycerol. Developmental time (DT) was estimated as the average number of hours from first-instar larva to adult emergence, where all females and males counted at a particular scoring were taken as having emerged at the midpoint in time between that scoring and the previous one. Whenever possible, up to four females and four males per vial were scored for their thorax length (TL). TL was measured to the nearest 0.013 mm from the anterior margin of the thorax to the posterior tip of the scutellum, as viewed laterally, using a binocular microscope fitted with an ocular micrometer. All measurements were made by one of us (EB). The numbers of pupae and adult flies were used to calculate the proportion of first-instar larvae successfully developing into pupae (VP) and into adult (VA).

Experiment B.—From approximately 300 individuals emerged from rotting *Opuntia* fruits collected at Colera, 100 isofemale strains were set up in November 1991. After two generations of identical rearing conditions to eliminate any environmental and residual nongenetic parental effects, 11 virgin males and 11 virgin females from each isofemale strain were mixed and randomly distributed into four plastic cages ($18 \times 14.5 \times 9.5$ cm^3). Eggs for the experiment were collected over a four-hour period on petri dishes containing nonnutritive agar with a generous smear of live yeast. After the egg-laying period, the petri dishes were incubated at 25°C for 26 h. Newly hatched first-instar larvae were collected using paintbrushes and transferred to vials (10.8-cm depth, 3-cm diameter) containing 20 mL of standard cornmeal-agar-yeast food. A total of 32 vials with 80 first-instar larvae each were produced and placed in the incubator at 23°C (12:12 L:D cycle) with uncontrolled humidity.

Emerging adults were counted every six hours, and a total of 1811 flies were raised from the experimental vials (i.e., an overall viability of ~71% was obtained). Developmental time (DT), thorax length (TL), and second-chromosome karyotype were recorded in a random sample of 255 males

and 205 females collected during the first five emerging days, when ~98% of the adults had emerged. This was done to avoid the potential inbreeding effects on DT and TL due to consanguineous matings in the egg-laying cages (about 1% if random mating is assumed). DT was estimated as the number of hours from egg-hatch to adult emergence (all females and males counted at a particular scoring were taken as having emerged at the midpoint in time between that scoring and the previous one). TL was measured as indicated above. The flies were subsequently assayed for their *Esterase-2* (*Est-2*) genotype by means of horizontal starch gel electrophoresis (Quezada-Díaz et al. 1992) to infer their karyotype. Previous observations in this population revealed a strong linkage disequilibrium between alleles at this locus and the second-chromosome inversions (Betrán et al. 1995). Thus, allele $Est\text{-}2^a$ is fixed within *2st*, while *2j* contains both $Est\text{-}2^a$ and $Est\text{-}2^b$, with the former at a lower frequency. Allele $Est\text{-}2^{c+}$ is fixed in $2jq^7$, and allele $Est\text{-}2^c$ is only present in $2jz^3$. When a fly was scored as $Est\text{-}2^{a/-}$, its karyotype was ascertained by analyzing the salivary gland chromosomes of eight offspring third-instar larvae (the individuals had been previously crossed with virgin flies from a laboratory stock homozygous for $2jz^3$). In this experiment, the three arrangements *2j*, $2jz^3$, and $2jq^7$ were pooled into a single class (denoted as *2j*●). These three arrangements share inversion *2j* and are thus cytologically derived compared to the *2st* arrangement (Ruiz et al. 1982, 1991).

Statistical Analyses

STATISTICA (StatSoft 1996), implemented in a 486 PC-compatible, and SAS (1989) for VAX/VMS were used for the statistical analyses. We also used MATHEMATICA (Wolfram 1991) to handle the SSP matrices to estimate the variances and covariances explained by the inversions (see below). All the data for DT and TL were *ln*-transformed before the analyses.

Experiment A.—Here we analyze the effects of the second-chromosome karyotype on viability, developmental time, and thorax length, together with their interaction with larval density. The unit of analysis in this experiment is the vial, which means that we had 600 (1200 if sex is included; see below) statistically independent observations for each of the traits studied. All viability data were transformed using the arcsine square-root transformation (Sokal and Rohlf 1995). To simplify the statistical analyses, the five replicates were standardized to the same global average. The model of analysis is:

$$y_{ijkl} = \mu + \alpha_i + C_{j(i)} + \beta_k + \gamma_l + \alpha\beta_{ik} + \alpha\gamma_{il} + C\beta_{jk} + C\gamma_{jl} + \beta\gamma_{kl} + \alpha\beta\gamma_{ikl} + C\beta\gamma_{jkl} + e_{ijkl}, \quad (1)$$

where μ is the overall grand mean, α_i is the fixed effect of the *i*th ($i = 1, 2, \ldots 10$) karyotype, $C_{j(i)}$ is the random effect of the independent inbred strains used in the crosses nested within karyotypes ($j = 1, 2, 3$), β_k is the fixed effect for the direction of cross (i.e., maternal effects; $k = 1, 2$), γ_l is the fixed effect for the larval density ($l = 1, 2$), and e_{ijkl} is the residual error associated with the corresponding viability value of the *ijkl*th vial. For the variables DT and TL the analyses also included sex as a fixed factor and, therefore, the linear model above had to be modified as to include sex and all possible interactions with this factor.

As previously noted, the arrangements *2j*, $2jz^3$, and $2jq^7$ share inversion *2j* and are derived compared to *2st*, which is the species ancestral arrangement (Ruiz et al. 1982). For this reason, the variation explained by the 10 karyotypes in the ANOVAs was further decomposed after pooling those three arrangements above into a single class (*2j*●) as follows: among *2st/2st*, *2st/2j*●, *2j*●*/2j*●; among the three *st/j*● heterokaryotypes; and among the six *j*●*/j*● karyotypes.

The karyotypic values for the different traits were also estimated in the additive-dominance scale (Mather and Jinks 1971). With this experimental design we could test for all six possible additive and dominance effects by means of linear contrasts. Thus, the additive effect of, for instance, *2st/2st* versus *2j/2j* homokaryotypes could be tested as the difference between the two means, and the dominance effect as the difference between the mean value of the *2st/2j* heterokaryotype versus the average of the corresponding values for the two homokaryotypes. However, to avoid multiple a priori comparisons we decided to test first for the effect of karyotypes after pooling the three arrangements that share inversion *2j*. If no statistically significant effects were detected in the ANOVAs among the three *st/j*● heterokaryotypes, and the six *j*●*/j*● karyotypes (see above), additive and dominance effects were only tested for *2st/2st* versus *2j*●*/2j*●, and *2st/2j*● versus the average of *2st/2st* and *2j*●*/2j*●, respectively (each comparison or contrast between two means has one degree of freedom).

MANOVAs were carried out assuming multivariate normality and homogeneity of variance-covariance matrices for the different groups. These are somewhat restrictive assumptions, but MANOVA seems to be robust to deviations from these assumptions when experimental designs are balanced, as in the present situation (Harris 1975).

We crudely approached the genetic correlations between DT and TL as follows (not be confounded with the multivariate genetic model discussed below for experiment B). Assuming that the components of the between karyotype SSP hypothesis matrix ($\mathbf{H}_k$) are entirely genetic in origin, the correlation coefficient between the means of all 10 karyotypes is given by

$$r_k = \frac{\mathbf{H}_k(1, 2)}{\sqrt{\mathbf{H}_k(1, 1)\mathbf{H}_k(2, 2)}}, \quad (2)$$

where $\mathbf{H}_k(1,2)$ is the off-diagonal element (sum of products of karyotypes averages), and $\mathbf{H}_k(i,i)$ is the diagonal element (sum of squares of karyotypes averages) for the *i*th variable. This correlation coefficient is obviously an approximation to the genetic correlation because the $\mathbf{H}_k$ matrix also contains a fraction of the variation among the strains used to obtain the tetrahybrid larvae (see above). The correlation coefficient can be tested as

$$t = r\sqrt{\frac{k - 2}{1 - r^2}}, \quad (3)$$

were *k* is the number of karyotypes (Sokal and Rohlf 1995). After pooling the three arrangements that share inversion *2j*

into a single class, we can now obtain the new hypothesis matrix, $\mathbf{H}_p$. The correlation coefficient between the pooled averages can be estimated as

$$r_p = \frac{\mathbf{H}_p(1, 2)}{\sqrt{\mathbf{H}_k(1, 1)\mathbf{H}_k(2, 2)}}. \quad (4)$$

The square of this correlation can be interpreted as that fraction of the total variation among karyotypes that is explained by *2st/2st, 2st/2j●*, and *2j●/2j●*. Similarly, we can estimate the correlation between the additive effects of *2st/2st* versus *2j●/2j●* from the corresponding hypothesis matrix ($\mathbf{H}_a$).

Experiment B.—The effects of the second-chromosome inversions on DT and TL were estimated using the multivariate genetic model proposed by Ruiz and Barbadilla (1995) to analyze the contribution of an arbitrary number of multiallelic loci upon any number of quantitative traits in a random mating population. The following is a summary of their model as applied to our particular situation.

Let us consider a single locus (chromosome) with a alleles (inversions). There will be $k = a(a + 1)/2$ different genotypes (karyotypes), $n = a - 1$ independent allelic doses, and $m = a\ (a - 1)/2$ independent allelic dose products. Then, the multivariate genetic model is:

$$\mathbf{z} = \boldsymbol{\alpha}^{\mathrm{T}}\mathbf{x} + \boldsymbol{\sigma}^{\mathrm{T}}\mathbf{y} + \mathbf{e}, \quad (5)$$

where $\mathbf{z}$ and $\mathbf{e}$ are (2×1) vectors of phenotypic values and environmental deviations (given as deviation from the population mean), respectively; $\boldsymbol{\alpha}$ and $\boldsymbol{\sigma}$ are $(n \times 2)$ and $(m \times 2)$ matrices of average effects of a gene (inversion) substitution and dominance departures of heterozygotes from the midpoint between the two homozygotes, respectively; $\mathbf{x}$ and $\mathbf{y}$ are $(n \times 1)$ and $(m \times 1)$ vectors of allelic doses and allelic dose products, respectively; and the superscript $^{\mathrm{T}}$ denotes transposition.

The phenotypic variance-covariance matrix, $\mathbf{P}$, can be decomposed into causal components of variance as follows:

$$\mathbf{P} = \mathbf{K} + \mathbf{E} = \mathbf{A} + \mathbf{D} + \mathbf{E}, \quad (6)$$

where $\mathbf{K}$, $\mathbf{A}$, and $\mathbf{D}$ are, respectively, the (2×2) diagonal variance-covariance matrices of genotypic (karyotypic), additive, and dominance contributions:

$$\mathbf{K} = \begin{bmatrix} \sigma_{k11} & \sigma_{k12} \\ \sigma_{k12} & \sigma_{k22} \end{bmatrix}, \quad \mathbf{A} = \begin{bmatrix} \sigma_{a11} & \sigma_{a12} \\ \sigma_{a12} & \sigma_{a22} \end{bmatrix}, \quad \mathbf{D} = \begin{bmatrix} \sigma_{d11} & \sigma_{d12} \\ \sigma_{d12} & \sigma_{d22} \end{bmatrix}. \quad (7)$$

Following Mode and Robinson (1959), the genotypic (karyotypic), additive, and dominance corelations can be defined as:

$$r_k = \frac{\sigma_{k12}}{\sqrt{\sigma_{k11}\sigma_{k22}}}, \quad r_a = \frac{\sigma_{a12}}{\sqrt{\sigma_{a11}\sigma_{a22}}}, \quad r_d = \frac{\sigma_{d12}}{\sqrt{\sigma_{d11}\sigma_{d22}}}, \quad (8)$$

where

$$\sigma_{k11} = \sigma_{a11} + \sigma_{d11} \qquad \sigma_{k22} = \sigma_{a22} + \sigma_{d22} \qquad \sigma_{k12} = \sigma_{a12} + \sigma_{d12}$$

$$\sigma_{a11} = \sum_{i=1}^{n} \alpha_{i1}^2\sigma_{x_i}^2 \qquad \sigma_{a22} = \sum_{i=1}^{n} \alpha_{i2}^2\sigma_{x_i}^2 \qquad \sigma_{a12} = \sum_{i=1}^{n} \alpha_{i1}\alpha_{i2}\sigma_{x_i}^2$$

$$\sigma_{d11} = \sum_{i=1}^{m} \delta_{i1}^2\sigma_{y_i}^2 \qquad \sigma_{d22} = \sum_{i=1}^{m} \delta_{i2}^2\sigma_{y_i}^2 \qquad \sigma_{d12} = \sum_{i=1}^{m} \delta_{i1}\delta_{i2}\sigma_{y_i}^2 \quad (9)$$

Those correlations measure the relative degree of similarity of the overall, additive, and dominance effects of the locus upon the two phenotypic traits.

In the particular case of a diallelic locus (e.g., when only arrangements *2st* and *2j●* are considered), $n = m = 1$. Then, it can be shown that the expected additive and dominance correlations are always equal to either $+1$ or -1 (if the locus makes a statistically significant contribution to the phenotypic variance of both quantitative traits). To see this intuitively, let us consider two alleles with additive effects on two traits. The effect of a gene substitution is ;α_1 on trait 1 and α_2 on trait 2. Therefore,

$$\sigma_{a12} = 2pq\alpha_1\alpha_2, \quad (10a)$$

$$\sigma_{a11} = 2pq\alpha_1^2, \quad (10b)$$

$$\sigma_{a22} = 2pq\alpha_2^2. \quad (10c)$$

The genetic correlation is:

$$r_a = \frac{2pq\alpha_1\alpha_2}{\sqrt{4p^2q^2\alpha_1^2\alpha_2^2}}, \quad (11)$$

and its value would be obviously dependent on the contribution of the alleles to the covariance. The karyotypic correlation, on the other hand, will vary between $+1$ and -1 depending on whether or not there are dominance effects of the locus upon the two traits.

The statistical model to obtain unbiased estimates of $\mathbf{K}$, $\mathbf{A}$, and $\mathbf{D}$ variance-covariance matrices in our case is the following:

$$\mathbf{Z}_N = \mathbf{X}_N\boldsymbol{\alpha} + \mathbf{Y}_N\boldsymbol{\sigma} + \boldsymbol{\epsilon}_N, \quad (12)$$

where $\mathbf{Z}_N$ is the $(N \times 2)$ data matrix whose row i comprises the observed values for the two measured quantitative traits DT and TL (expressed as deviations from the mean), respectively, on the ith individual; $\mathbf{X}_N$ and $\mathbf{Y}_N$ are $(N \times 1)$ vectors of individual allelic dose and dose products, respectively; and $\boldsymbol{\epsilon}_N$ is the $(N \times 2)$ matrix of random errors. The statistical model, therefore, allows to split the total matrix $\mathbf{T}$ of sums of squares and cross products into three orthogonal components as follows:

$$\mathbf{T} = \mathbf{H}_k + \mathbf{R} = \mathbf{H}_a + \mathbf{H}_d + \mathbf{R}, \quad (13)$$

where $\mathbf{H}_k$ is the SSP matrix explained by the model, $\mathbf{H}_a$ is the SSP matrix explained by the allelic doses, $\mathbf{H}_d$ $(= \mathbf{H}_k - \mathbf{H}_a)$ is the SSP matrix explained by the dose products, and $\mathbf{R}$ is the SSP residual matrix. The matrices with the causal variance-covariance components are then obtained as:

$$\mathbf{K} = \frac{\mathbf{H}_k - (k-1)\mathbf{E}}{N}, \quad (14a)$$

$$\mathbf{A} = \frac{\mathbf{H}_a - (a-1)\mathbf{E}}{N}, \quad (14b)$$

$$\mathbf{D} = \frac{\mathbf{H}_d - (k-a)\mathbf{E}}{N}, \quad (14c)$$

where

$$\mathbf{E} = \frac{\mathbf{R}}{N - (k-1) - 1}.$$

Statistical significance of the diagonal effects (variances) in these matrices can be tested by the corresponding ANOVAs (Ruiz and Barbadilla 1995). The off-diagonal elements (covariances) can be tested by means of the karyotypic, additive, and dominance correlations using a *t*-test. For instance, for the karyotypic correlation coefficient:

$$t = \frac{\hat{r}_k}{\hat{s}_{r_k}}, \quad (15)$$

where $\hat{s}_{r_k}$ is the sampling standard deviation of the correlation coefficient, with $N - (k-1) - 2$ degrees of freedom. The sampling variance of the karyotypic correlation can be obtained as (see Kendall and Stuart 1969, p. 235):

$$\mathrm{Var}(\hat{r}_k) = \hat{r}_k^2\left[\frac{\mathrm{Var}(\sigma_{k12})}{\sigma_{k12}^2} + \frac{\mathrm{Var}(\sigma_{k11})}{4\sigma_{k11}^2} + \frac{\mathrm{Var}(\sigma_{k22})}{4\sigma_{k22}^2} + \frac{\mathrm{Cov}(\sigma_{k11},\sigma_{k12})}{\sigma_{k11}\sigma_{k12}} + \frac{\mathrm{Cov}(\sigma_{k22},\sigma_{k12})}{\sigma_{k22}\sigma_{k12}} + \frac{\mathrm{Cov}(\sigma_{k11},\sigma_{k22})}{2\sigma_{k11}\sigma_{k22}}\right]. \quad (16)$$

The sampling (co)variances between the elements of matrix **K** can be estimated using the following formula based on Mode and Robinson (1959) and Searle et al. (1992):

$$Cov(\sigma_{kij},\sigma_{kqr}) = \left(\frac{k-1}{N}\right)^2\left(\frac{\mathbf{M}_{k(iq)}\mathbf{M}_{k(jr)} + \mathbf{M}_{k(ir)}\mathbf{M}_{k(jq)}}{k+1} + \frac{\mathbf{E}_{(iq)}\mathbf{E}_{(jr)} + \mathbf{E}_{(ir)}\mathbf{E}_{(jq)}}{N-k+2}\right), \quad (17)$$

where σ_{kij} and σ_{kqr} are, respectively, elements (i, j) and (q, r) of the matrix **K**, $\mathbf{M}_{k(iq)}$ is element (i, q) of the matrix $\mathbf{M}_k$ (= $\mathbf{H}_k/[k-1]$), and $\mathbf{E}_{(iq)}$ is the element (i, q) of the matrix **E**.

A similar procedure can be used to test the additive and dominance correlation coefficients. The degrees of freedom of the *t*-test are $N - (a-1) - 2$ in the former case, and $N - (k-a) - 2$ in the latter.

The procedures depicted here to test the off-diagonal elements of matrices **K**, **A**, and **D** differ from those outlined in Ruiz and Barbadilla (1995), which do not seem to us to be fully satisfactory.

Results

Experiment A

A MANOVA was carried out to test for the different factors affecting the larva-to-pupae viability (VP) and the larvae-to-adult viability (VA) (results not shown). The only statistically significant linear effects were those arising from genetic differences among the inbred strains used to build up the 10 possible karyotypes (Wilks lambda = 0.870, $P = 0.004$; see Materials and Methods), and the growth densities of the larvae (Wilks lambda = 0.395, $P < 0.001$). The corresponding ANOVAs revealed that the two viabilities were significantly lower at the high growth density (VP = 0.787 vs. 0.815; F = 29.53, $P < 0.001$, and VA = 0.688 vs. 0.720; F = 9.82, $P = 0.005$). In no instances were the average viabilities for the second-chromosome karyotypes, either pooled or not, statistically different. For VP and VA , the linear contrasts revealed lower values for *2j●/2j●* when compared to *2st/2st*, and the heterokaryotype *2st/2j●* was always close to the average of *2st/2st* and *2j●/2j●*, but the differences were not statistically significant (data not shown).

The MANOVA for DT and TL revealed statistically significant differences among karyotypes and sexes, in addition to the previously observed differences among inbred strains and growth densities for the two viabilities (Table 1). The differences among karyotypes were basically due to the variable DT, because no statistically significant effect of karyotype on TL was observed, and the effects were additive. However, a strong karyotype × sex interaction for TL was detected, and genotypic values were obtained separately for females and males after pooling for the two growth densities. This was justified because no statistically significant karyotype × sex × density interaction was observed (Table 1). In addition, the growth density × sex statistically significant interaction simply reflects the fact that sexual dimorphism for TL decreased at the high growth density.

Figure 1 plots the genotypic values in the additive-dominance scale for DT and TL, together with their statistical significance obtained from the a priori planned comparisons. Because no statistically significant effects were observed among the three heterokaryotypes *st/j●*, or among the six karyotypes *j●/j●* (Table 1), additive and dominance effects were only tested after pooling the three arrangements that share inversion *2j* (see Materials and Methods). It is clear that the homokaryotype *2st/2st* consistently decreased both DT and TL when compared to the *2st/2j●* and *2j●/2j●* pooled karyotypes. In all cases but one (TL in males) additive effects were statistically significant, and the heterokaryotype *2st/2j●* was always close to the midparent (i.e., dominance was not statistically significant).

Also evident from Figure 1 is the positive contribution that the second-chromosome karyotypes make to the correlation between DT and TL in both sexes. The karyotypic correlations (see Materials and Methods) were: $r_k = 0.792$ ($P = 0.003$) and $r_p = 0.619$ ($P = 0.028$) for females; and $r_k = 0.174$ ($P = 0.316$) and $r_p = 0.069$ ($P = 0.425$) for males. Most of the correlation in females can be attributed to the additive effects ($r_a = 0.574$; $P = 0.041$). As previously argued, the observed correlation of karyotype means might provide little indication of the correlation unless we can as-

TABLE 1. MANOVA, together with the corresponding ANOVAs, for DT (developmental time from first-instar larva to adult) and TL (thorax length) measured for the 10 different second-chromosome karyotypes at the two larval densities in experiment A (see text for details). Significant values ($P < 0.05$) are indicated by boldface type.

	MANOVA				ANOVAs					
							DT		TL	
Effect	Wilks' lambda	df 1	df 2	P	df effect	df error	F	P	F	P
Karyotypes (1)	**0.277**	18	38	0.047	9	20	**3.05**	0.018	0.50	0.858
2st/2st, 2st/2j•, 2j•/2j•	**0.591**	4	38	0.037	2	20	**6.39**	0.007	1.71	0.207
additive effect	**0.654**	2	38	0.018	1	20	**10.02**	0.005	2.91	0.104
dominance effect	0.997	2	38	0.976	1	20	2.77	0.112	0.51	0.485
Among *st/j•*	0.732	4	38	0.194	2	20	1.10	0.351	0.15	0.860
Among *j•/j•*	0.451	10	38	0.082	5	20	2.50	0.065	0.16	0.976
Lines (2)	**0.688**	40	1918	< 0.001	20	960	**9.72**	< 0.001	**12.34**	< 0.001
Maternal effect (3)	0.868	2	19	0.261	1	20	2.21	0.153	1.16	0.295
Growth density (4)	**0.006**	2	19	< 0.001	1	20	**7.12**	0.015	**3028.63**	< 0.001
Sex (5)	**0.003**	2	19	< 0.001	1	20	**117.23**	< 0.001	**7274.16**	< 0.001
1 × 3	0.401	18	38	0.292	9	20	2.23	0.065	0.62	0.770
1 × 4	0.456	18	38	0.466	9	20	1.09	0.409	0.81	0.612
1 × 5	0.291	18	38	0.063	9	20	0.61	0.772	**3.62**	0.008
2 × 3	**0.910**	40	1918	< 0.001	20	960	1.44	0.095	**3.22**	< 0.001
2 × 4	**0.931**	40	1918	0.003	20	960	1.23	0.219	**2.34**	< 0.001
2 × 5	0.977	40	1918	0.988	20	960	0.51	0.962	0.62	0.902
3 × 4	0.902	2	19	0.374	1	20	0.40	0.535	1.50	0.234
3 × 5	0.868	2	19	0.260	1	20	2.47	0.132	0.03	0.861
4 × 5	**0.335**	2	19	< 0.001	1	20	1.61	0.219	**35.22**	< 0.001
1 × 3 × 4	0.556	18	38	0.769	9	20	0.76	0.654	0.79	0.630
1 × 3 × 5	0.484	18	38	0.559	9	20	0.54	0.827	1.26	0.315
1 × 4 × 5	0.335	18	38	0.130	9	20	1.31	0.293	1.89	0.113
2 × 3 × 4	0.948	40	1918	0.109	20	960	0.77	0.754	**1.74**	0.023
2 × 3 × 5	0.983	40	1918	0.999	20	960	0.43	0.987	0.44	0.984
2 × 4 × 5	0.979	40	1918	0.996	20	960	0.62	0.901	0.36	0.996
3 × 4 × 5	0.977	2	19	0.802	1	20	0.47	0.502	0.03	0.871
1 × 3 × 4 × 5	0.404	18	38	0.300	9	20	1.11	0.397	1.48	0.223
2 × 3 × 4 × 5	0.977	40	1918	0.989	20	960	0.41	0.990	0.66	0.869

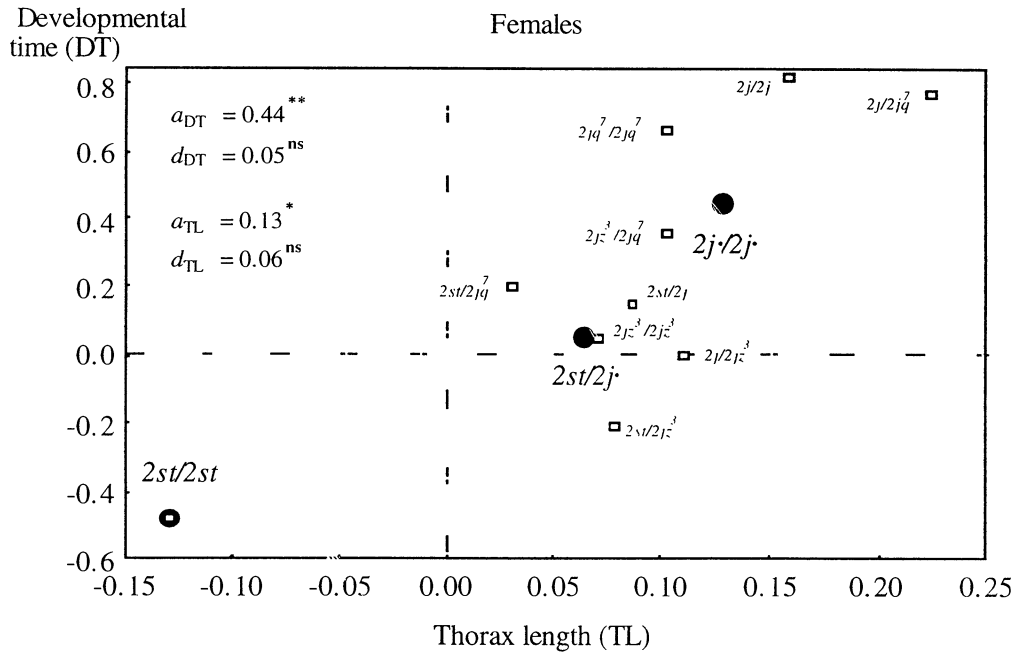
sume that the environmental covariance is small (Kempthorne 1969, pp. 264–267). However, it is also quite clear that as far as a diallelic locus makes a statistically significant contribution to the phenotypic variance of both traits (Fig. 1), the expected additive correlation will be either +1 or −1 (see above). Therefore, the results do suggest that second-chromosome inversions in *D. buzzatii* make a positive contribution to the genetic correlation between DT and TL.

Experiment B

Summary statistics for each trait are given in Table 2. An exact test for genetic differentiation (Raymond and Rousset 1995) did not detect heterogeneity in inversion frequencies between sexes ($P = 0.730$). These frequencies were 0.1630 for *2st*, and 0.8370 for *2j•*, in good agreement to the previously obtained values in the same population at Colera (Betrán et al. 1995). Because Ruiz and Barbadilla (1995) statistical approach assumes a random mating population, exact Hardy-Weinberg tests were performed for each sex (Rousset and Raymond 1995). No departures from the expected proportions were detected in any case ($F_{IS} = -0.026$, $P = 0.807$ for females; and $F_{IS} = 0.019$, $P = 0.814$ for males).

A positive and statistically significant phenotypic correlation between DT and TL was observed for both females ($r = 0.220$, $P = 0.002$) and males ($r = 0.164$, $P = 0.009$). A MANOVA test considering the karyotype and sex as fixed effects did not detect a statistically significant interaction between these two factors (Wilks lambda = 0.997, $P = 0.821$), and further analyses were carried out grouping both sexes after multiplying females values by correcting factors to make their averages equal to those of males (males took ~1.2% more time to develop and were ~10.8% smaller than females). Table 3 gives the MANOVA, and the corresponding ANOVAs, for the effects of karyotype on DT and TL, together with the regression on the chromosome dose and the chromosome dose products (deviations). Also shown in Table 3 are the estimated variance-covariance matrices explained by the karyotypes (**K**), together with its decomposition into additive (**A**) and dominance (**D**) components, and the error matrix (**E**). Chromosome inversions affect significantly both variables, with the effects being mainly additive. The contribution of the chromosomes to the karyotypic and additive covariances between the two traits were, respectively, $\hat{r}_k = 0.897$ (approximate 95% confidence limits: 0.405–1.389) and $\hat{r}_a = 1.155$ (approximate 95% confidence limits: 0.849–1.461). Note that in our case r_a (and r_k) can take one out of its only two theoretical values: either +1 or −1 (see Material and Methods). Both correlations are higher than zero and the 95% confidence limits include their expected value. The conclusion is, therefore, that the second-chromosome inversions make a positive contribution to the total genetic correlation between DT and TL.

Average effects of second-chromosome inversions on both traits, estimated as the regression coefficients to the chro-



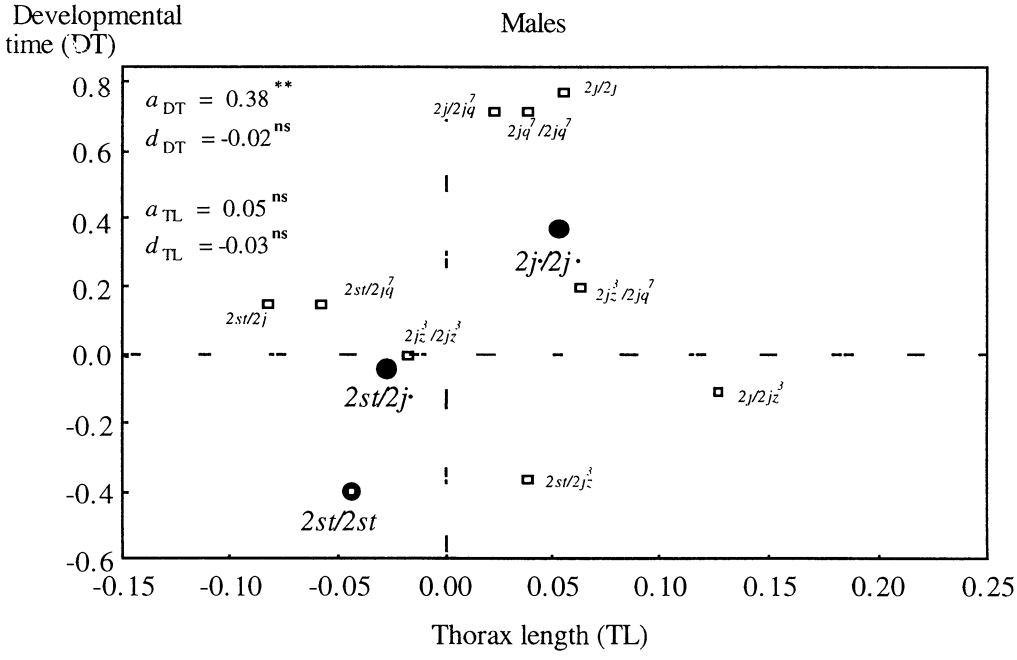


FIG. 1. Karyotypic values, in the additive-dominance scale, for the variables developmental time (DT; in *ln* h) and thorax length (TL; in *ln* mm) in the experiment A. All deviations were measured after pooling the three arrangements *2j*, $2jz^3$, and $2jq^7$ into a single class denoted as *2j*• and the coordinate point (0,0) was taken as the midparent (i.e., the average of TL and DT for the two homokaryotypes *2st/2st* and *2j•/2j•*. In the original scale without *ln*-transformation, this point corresponds to an average TL of 1.111 mm for females and 1.034 mm for males, and an average DT of 335.3 h for females and 340.9 h for males. Open squares give the values for all 10 karyotypes to appreciate their dispersion from the midparent, as well as their dispersion from the pooled *2st/2j•* and *2j•/2j•* karyotypes (black circles). Additive (a_{DT} and a_{TL}) and dominance (d_{DT} and d_{TL}) effects were tested for statistical significance as indicated in Materials and Methods (ns = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$).

TABLE 2. Mean values of developmental time (h) and thorax length (mm), together with their standard deviations (SD), for the second-chromosome karyotypes (arrangements *2j, 2jz*^{3}, and *2jq*^{7} were pooled into a single class denoted as *2j•*) in the laboratory females and males of *Drosophila buzzatii* raised from wild flies collected at Colera (experiment B; see text for details).

Trait	Sex		Karyotype *2st/2st*	Karyotype *2st/2j•*	Karyotype *2j•/2j•*	Total
Developmental time	Females	Mean ± SD	319.6 ± 18.3	310.3 ± 19.4	315.5 ± 20.5	314.1 ± 20.2
		n	5	59	141	205
	Males	Mean ± SD	314.9 ± 22.3	314.6 ± 18.1	319.0 ± 19.0	317.7 ± 18.8
		n	7	67	181	255
Thorax length	Females	Mean ± SD	1.176 ± 0.055	1.187 ± 0.029	1.199 ± 0.026	1.195 ± 0.029
		n	5	59	141	205
	Males	Mean + SD	1.059 ± 0.024	1.075 ± 0.024	1.081 ± 0.023	1.079 ± 0.024
		n	7	67	181	255

mosome dose, were: for TL (in *ln* mm), −0.0087 for *2st* and 0.0017 for *2j•*; for DT (in *ln* h), −0.0107 for *2st* and 0.0021 for *2j•*. Statistical significance for these average effects is obtained from the corresponding ANOVAs in Table 3.

DISCUSSION

Our results corroborate and extend earlier work with *D. buzzatii* showing that the second-chromosome polymorphic inversions have an additive effect on TL and other size-related biometrical traits (Table 4). Some experiments (e.g., our experiment B) follow the Edinburgh-Ames school of quantitative genetics (Kempthorne 1969; Falconer and Mackay 1996). In these experiments, the average effects of genes (or chromosomal arrangements), which are functions of both the gene action and the allelic (arrangement) frequencies can be estimated from the regression of the phenotypic values to the allelic doses (Ruiz and Barbadilla 1995). Other experiments (e.g., our experiment A) were inspired by the Birmingham school (Mather and Jinks 1971) and used inbred lines and their crosses. The results from the latter experiments only provide estimates of gene action and do not allow inferences to the base populations. It is possible, however, to obtain estimates of the average effects, or "statistically additive effects," by taking into account the gene action and the arrangement frequencies in the natural populations (Falconer and Mackay 1996; see Table 4). The different studies are in remarkably good agreement: in both sexes the species ancestral arrangement *2st* decreases body size when compared to the derived *2j•* arrangements (Table 4). In *D. buzzatii* polymorphic inversions are an important source of genetic variation for TL.

The new findings are that larval DT is also (additively) affected by the second-chromosome inversions, and that the additive genetic correlation between DT and TL explained by the inversions is positive. Here again the results are consistent across experiments: those larvae bearing the *2st* arrangement have, as an average, a shorter DT than those carrying the *2j* inversion (Table 4). The faster developmental time of larvae bearing *2st* should in theory allow this arrangement to gain a demographic advantage in increasing or colonizing populations such as those of *Drosophila* (e.g., Cole 1954; Lewontin 1965).

Although larval density had important effects on the expression of DT and TL, it had no influence on genetic effects as no karyotype × density statistically significant interaction was detected in experiment A (Table 1). There was no evidence that the *2st/2st* larvae, with a lower developmental time than both *2st/2j•* and *2j•/2j•* (Fig. 1), had a higher preadult survival at any growth density. While the pattern was in the "expected" direction in that the higher TL and longer DL of karyotypes with *2j•* seemed to be associated with a lower preadult survival (see above), as has been observed in *D. melanogaster* when artificially selected lines for large TL were compared to their control counterparts under high larval

TABLE 3. One-way MANOVA, together with the corresponding ANOVAs, for developmental time (DT; in *ln* h) and thorax length (TL; in *ln* mm) measured for the three different second-chromosome karyotypes in experiment B (arrangements *2j, 2jz*^{3}, and *2jq*^{7} were pooled). Also given are the matrices of estimated variance-covariance (all values × 10^4) explained by the karyotypes (**K**), by the chromosome does (**A**), and by the chromosome dose product (**D**), and the error matrix (**E**). The statistical significance for the diagonal elements (variances) in these matrices are given in the corresponding ANOVAs, and statistical significance for the off-diagonal elements (covariances) were obtained as indicated in Materials and Methods. Significant values ($P < 0.05$) are indicated by boldface type.

	MANOVA			ANOVAs				
					DT		TL	
Source of variation	df	Wilks' lambda	*P*	df	*F*	*P*	*F*	*P*
Karyotypes (K)	4	**0.951**	< 0.001	2	**3.09**	0.047	**9.60**	< 0.001
Regression (A)	2	**0.957**	< 0.001	1	**4.17**	0.042	**18.82**	< 0.001
Deviations (D)	2	0.994	0.247	1	2.00	0.158	0.40	0.526
Error (E)	912			457				

$$\mathbf{K} = \begin{bmatrix} & \text{DT} & \text{TL} \\ \text{DT} & \mathbf{0.32195} & \mathbf{0.22065} \\ \text{TL} & \mathbf{0.22065} & \mathbf{0.18782} \end{bmatrix}; \quad \mathbf{A} = \begin{bmatrix} & \text{DT} & \text{TL} \\ \text{DT} & \mathbf{0.24463} & \mathbf{0.25184} \\ \text{TL} & \mathbf{0.25184} & \mathbf{0.19433} \end{bmatrix}; \quad \mathbf{D} = \begin{bmatrix} & \text{DT} & \text{TL} \\ \text{DT} & 0.07732 & -0.03119 \\ \text{TL} & -0.03119 & -0.00651 \end{bmatrix}; \quad \mathbf{E} = \begin{bmatrix} & \text{DT} & \text{TL} \\ \text{DT} & 35.449 & 2.35379 \\ \text{TL} & 2.35379 & 5.02259 \end{bmatrix}$$

TABLE 4. Average effects of second-chromosome arrangements on thorax length (mm), size-related traits, and developmental time (h) in *Drosophila buzzatii*. Arrangements *2j*, *2jz*^{3}, and *2jq*^{7} have been grouped into a single class (*2j*•) for simplicity. Statistically significant values ($P < 0.05$) are indicated by boldface type.

Trait	Population	Sample	Frequency[1]		Average effects		Author
			2st	*2j*•	*2st*	*2j*•	
Thorax length	Carboneras (Spain)	Laboratory males (1987)	0.3815	0.6185	**−0.0054**	**0.0033**	Ruiz et al. (1991)
		Field solitary males (1987)	0.4109	0.5891	**−0.0059**	**0.0041**	Ruiz et al. (1991)
		Field mating males (1987)	0.3795	0.6205	**−0.0078**	**0.0048**	Ruiz et al. (1991)
		Field solitary females (1987)	0.4283	0.5717	−0.0014	0.0010	Ruiz et al. (1991)
		Field mating females (1987)	0.4281	0.5719	−0.0016	0.0012	Ruiz et al. (1991)
		Laboratory males (1989)	0.4564	0.5436	**−0.0052**	**0.0032**	Barbadilla (1992)
		Field males (1989)	0.4586	0.5414	**−0.0081**	**0.0068**	Barbadilla (1992)
		Field males (1990)	0.4124	0.5876	**−0.0144**	**0.0101**	Barbadilla (1992)
		Field gravid females (1990)	0.4457	0.5543	−0.0022	0.0018	Barbadilla (1992)
		Field ungravid females (1990)	0.5000	0.5000	−0.0041	0.0041	Barbadilla (1992)
		Males	0.4102	0.5898	−0.0027	0.0019	This work
		Females	0.4102	0.5898	**−0.0056**	**0.0039**	This work
	Colera (Spain)	Males + Females	0.1630	0.8370	**−0.0093**	**0.0018**	This work
	Arroyo Escobar (Argentina)	Males	0.1240	0.8760	**−0.0205**	**0.0029**	Hasson et al. (1992)
		Females	0.1240	0.8760	**−0.0143**	**0.0020**	Hasson et al. (1992)
Size-related traits	Arroyo Escobar (Argentina)	Field emerged flies (1989 and 1990)	0.1566	0.8434	**negative**	**positive**	Norry et al. (1995)
Developmental time	Carboneras (Spain)	Males	0.4102	0.5898	**−3.8233**	**2.6560**	This work
		Females	0.4102	0.5898	**−4.3640**	**3.0351**	This work
	Colera (Spain)	Males + Females	0.1630	0.8370	**−3.3898**	**0.6601**	This work

[1] Frequencies for Arroyo Escobar are from Hasson et al. (1995); those for Carboneras in this work from Ruiz et al. (1991, p. 744).

densities (Santos et al. 1992a; Partridge and Fowler 1993), the differences were not statistically significant. Some caution, however, should be taken in equating a faster developmental time and a smaller adult body size with a higher preadult survival under larval crowding.

There is conflicting evidence about the importance of larval density on the evolutionary responses of body size and developmental time in *Drosophila*. Thus, Roper et al. (1996) have reported that low density selection lines evolved extended development times and greater adult body size when compared to high density selection lines. On the other hand, Santos et al. (1997) showed that larval developmental time of populations subjected to extreme larval crowding was about the same as that of their corresponding control populations, but crowded populations did evolve a higher growth rate during the postcritical period of larval life. The differences in growth rate, however, did not translate into the adult stage and no differences in adult body size between treatments were observed. The authors suggest that evolution in a crowded larval environment does not result in genetically faster larvae and smaller adults, but in a lower efficiency of larvae at utilizing food to complete development (see also Joshi and Mueller 1996). The "high" density regime in Roper et al. (1996) was 150 larvae per vial, whereas in Santos et al. (1997) was ~1000–1500 larvae per vial. Previous results with the populations used in Santos et al. (1997) suggest that a selection treatment of 150 larvae per vial does not mimic the evolutionary outcomes of a truly crowded environment, which might explain these contradictory findings (L. D. Mueller, pers. comm. 1996). Because in *Drosophila* there are two periods of larval growth, a flexible precritical period and a fixed postcritical period (Bakker 1959; Robertson 1963; Santos et al. 1997), the relationships between larval developmental time, adult body size and preadult survival may not be easily predictable.

The ultimate goal of this research program in *D. buzzatii* is obviously to understand the processes involved in the maintenance of the inversion polymorphism in the wild. This species can be collected in nature throughout its life cycle, and selection-component analyses have been carried out in two natural populations (Ruiz et al. 1986; Hasson et al. 1991; Barbadilla et al. 1994). These studies revealed a pattern of endocyclic selection, that is, opposing directional selection episodes on gene arrangement frequencies during the life cycle. In addition, wild mating males have been found to be consistently larger than non-mating or single males (Santos et al. 1988; 1992b; Leibowitz et al. 1995), which suggested that this episode of selection might be countered by the putative advantage of faster developing larvae that produce genetically smaller adults, as found in *D. melanogaster* (Wilkinson 1987). Because the gene arrangement *2st* was known to be associated to a smaller adult size, it was clear that any progress in linking *D. buzzatii* life history with the polymorphic inversions had to focus on the effects of gene arrangements on larval developmental time and preadult viability. The experiments reported here suggest appreciable antagonistic pleiotropic effects of second-chromosome inversions on early (an increase in DT goes in the direction of lowered fitness) and late (TL) fitness-related characters. Whether these antagonistic effects explain the maintenance

of the inversion polymorphism can only be determined by additional studies.

Is there a trade-off between TL and DT in natural populations of *Drosophila*? Before discussing this question, it should be stressed that our results do not provide quantitative estimates of genetic parameters in the base populations. Apart from the fact that intentional inbreeding to obtain the isogenic lines used in experiment A has probably purged the genetic load of the population, the additive variances contributed by the second-chromosome inversions to DT and TL are minimum estimates, and the corresponding additive covariances may be larger than, equal to, or lower than the actual contributions of the putative QTLs that are in linkage disequilibrium with the inversions (Ruiz et al. 1991; Ruiz and Barbadilla 1995). In addition, the contribution of the inversions to the genetic correlation between DT and TL is likely to be a fraction of the total genetic correlation and may not arise from pleiotropic effects of the same genes, but could be due to different sets of genes that happen to be in linkage disequilibrium with the inversions. The observed positive genetic correlation between DT and TL, therefore, should only be taken as suggestive additional evidence that supports the previously reported trade-off between these two traits in *Drosophila* (Robertson 1960, 1963; Wilkinson 1987; Partridge and Fowler 1993; Santos et al. 1994; Nunney 1996).

Recent experiments with *D. buzzatii* do question the simple interpretation that body size reflects an evolutionary compromise between the conflicting effects of genetic variation on larval and adult performances. Leibowitz et al. (1995) have addressed whether the phenotypic differences between wild-caught mating and single males could be attributed to genetic differences between the samples. Though the laboratory-reared offspring of the mating males were on average larger (but not significantly so), less phenotypically variable, and developed faster than those of single males, their results did not provide clear evidence for directional selection acting on the genetic component of body size. They observed a negative genetic correlation between size and developmental time in the unmated males, a finding that is clearly inconsistent with what we would expect if there were antagonistic pleiotropy. Taken together, their results suggest that mating males carry a lower than average frequency of generally deleterious mutations that affect body size, mating success, and developmental time. On the other hand, Santos (1996) has shown that a males body size has no impact on mating success in competition among males that grew up under uncrowded conditions, but that size is important among males that grew up under crowded conditions. The phenotypic directional selection on size, however, did not translate into a genetic response when the distributions of body size among the offspring were compared.

Certainly, it is not clear whether there is a causal relationship between adult body size and male mating success in natural populations of *D. buzzatii*. Nonetheless, body size may still be important for other adult fitness characters known to be positively correlated with it such as longevity and female fecundity (Santos et al. 1992b; Partridge and Fowler 1993, and references therein), and the suggestion that there is a trade-off between body size and larval developmental time may still be an adequate guide to understand the evolutionary causes for the maintenance of the inversion polymorphism in *D. buzzatii*.

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