

Negative genetic correlation between traits of the *Drosophila* head, and interspecific divergence in head shape

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For morphological traits that are negatively correlated, the genetic correlation (r_g) between them might strongly influence patterns of morphological divergence and shape. Here, the pattern of divergence between two sibling species of cactophilic *Drosophila*, *D. buzzatii* and *D. koepferae*, is examined for two traits that are known to be negatively correlated in other *Drosophila* species: face width (FW) and width of both eyes (EW). Head width (HW, the sum of FW and EW, i.e. the total width of the head capsule) was also examined. Genetic and phenotypic correlations were estimated in the laboratory G2 generation of a sample of wild *D. buzzatii* derived from a population where *D. koepferae* is not present. Phenotypic correlations were also estimated in *D. buzzatii* and *D. koepferae* from another, very different, population where the species are sympatric. Consistent with studies in other *Drosophila* species, r_g was negative and significant for the correlation between FW and EW, and positive (but nonsignificant at a matrix-wide P -value of 0.05) for the correlations of HW with both FW and EW. This well-defined correlation pattern was also consistent with the phenotypic correlations in both *D. buzzatii* and *D. koepferae*. No significant difference in these traits was detected between *D. buzzatii* populations, but head shape has diverged between *D. buzzatii* and *D. koepferae*. Specifically, the two negatively correlated traits, FW and EW, have evolved in opposite directions in these two species, with HW showing no significant interspecific difference. The overall picture of this divergence pattern shows a striking concordance with the present evidence of negative correlations between FW and EW, and is consistent with the notion of r_g -related constraints on the pattern of interspecific differentiation.

Keywords: developmentally related traits, *Drosophila* head, interspecific divergence, morphology, negative genetic correlation, phenotypic correlation.

Introduction

Genetic correlations (r_g) between traits, which arise from pleiotropy or linkage relations among genes controlling the traits, are often considered as factors that affect directions and rates of short-term phenotypic evolution. The short-term response to selection depends not only on the heritabilities of the selected traits but also on the genetic and phenotypic covariances among traits (Falconer, 1989; Roff, 1997; Lynch & Walsh, 1998). In addition, r_g between pleiotropically related traits can be interpreted as a constraint on their joint microevolution (e.g. Cheverud, 1984; Arnold, 1992). Negative correlations between traits may be of considerable importance in morphological evolution, because negatively correlated

traits are at least liable to evolve in opposite directions, resulting in a substantial change in shape.

Genetic correlations between morphological traits are more often positive than correlations between other traits (Roff, 1996, 1997), but not all morphological traits are positively correlated. For instance, in developmentally related traits, negative r_g values are expected when the morphological traits arise through a subdivision of a developmental precursor into variable parts (Riska, 1986). Examples of this might be expected in the development in *Drosophila*, because the imaginal discs, which give rise to the organs and external morphology of the adult fly after metamorphosis, are subdivided into compartments (García-Bellido *et al.*, 1973; Crick & Lawrence, 1975).

The head capsule is a body part that has received little attention in quantitative studies with *Drosophila*

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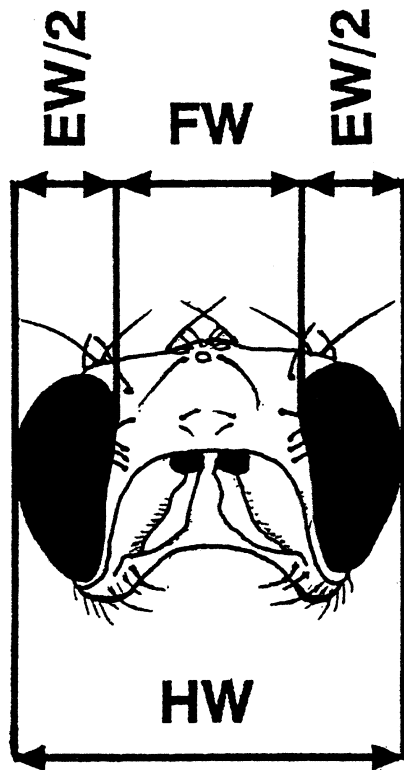


Fig. 1 Description of each measured trait. FW, face width; EW, eye width; HW, head width.

(but see Templeton, 1977; Vall, 1977; Cowley & Atchley, 1988), at least when compared with the growing research on the quantitative genetics of the wings. This body part is largely produced by a single pair of imaginal discs (the eye-antennal discs, Morata & Lawrence, 1979), and two traits derived from this developmental precursor, face width and eye width (Fig. 1), are negatively genetically correlated in *D. melanogaster* (see Cowley's results discussed in Riska, 1986; p. 1307; Cowley & Atchley, 1990).

Here, the cactophilic fly *D. buzzatii*, and its sibling species, *D. koepferae*, are examined for these developmentally related traits. Several aims are addressed. First, we estimate heritabilities and genetic correlations among face width, eye width and head width (Fig. 1) in the laboratory G2 generation of a sample of wild *D. buzzatii*. Face width is of special interest in this species because males with wider faces have a mating advantage (Norry *et al.*, 1995; Norry & Vilardi, 1996). Secondly, we estimated phenotypic correlations in both *D. buzzatii* and *D. koepferae*, addressing the hypothesis that phenotypic correlations among these traits are similar, in sign, to their genetic counterparts. Finally, we examined whether the two negatively correlated traits, face width and eye width, have evolved in opposite directions in these two sibling species.

Materials and methods

Experimental design to estimate genetic parameters

Genetic parameters were estimated in laboratory-reared flies of *D. buzzatii* derived from an Argentinian population breeding on *Opuntia vulgaris* at Arroyo Escobar (34°4'S, 58°7'W), Province of Buenos Aires, where *D. koepferae* is absent.

Wild females collected over banana baits (mid-March 1993) were individually kept in 95 × 20-mm shell vials containing 5 mL of David's (1962) medium (hereafter referred to as *standard vials*), obtaining 70 isofemale lines. Seven mating groups were established. Each group was obtained by releasing one male and one virgin female from the G1 of each isofemale line (140 flies) into a plastic chamber containing two 75-mm-diameter Petri dishes with an egg-laying medium (15 g agar, 75 mL 95% ethanol, 15 mL glacial acetic acid in 1500 mL water). Food was available in small containers. After 48–72 h, samples of 30 eggs were collected from each chamber and transferred to *standard vials*. Two sets of these vials were obtained for each mating group. Virgin flies eclosing from these cultures were paired at random by using a balanced 14 × 14 factorial design (196 pairs). Each resulting pair was kept in a vial with fresh food for 48 h prior to transferring both individuals to a 20 × 30-mm vial containing egg-laying medium. After 36–48 h both adults were removed and preserved at –20°C for measurement, and after 72–96 h, a sample of 30 first instar larvae was collected from vials containing sufficient larvae (114 out of 196 vials) and transferred to a *standard vial*. All eclosing offspring were collected (9 vials were discarded due to problems in the cultures), and the parental pairs and four offspring (two flies of each sex) were measured. A total of 105 families that were reared under standardized conditions of larval density were thus obtained. Throughout the experiment, cultures were reared at 24 ± 1°C.

The above procedure resulted in an effective random mating (the Pearson correlation between sires and dams was positive but nonsignificant for the traits described in Fig. 1, with the highest *r*-value for EW: *r* = 0.15; d.f. = 103). All measurements were performed with a binocular microscope fitted with an ocular micrometer. To measure each trait, the head was removed and observed from the front (Fig. 1) at 100× magnification (1 mm = 80 ocular units). Eye width (EW, width of both eyes) was considered as the sum of both measures, that of the left eye and that of the right eye. Previous studies showed that another related trait, eye length, is positively correlated with both FW and EW (Cowley &

Atchley, 1990; Norry *et al.*, 1997). Because we are concerned not with positive correlations but with negatively correlated traits, data on this trait were omitted in the present study.

Collection of strains derived from the Quilmes population

The same traits were also scored in males of *D. buzzatii* and *D. koepferae* derived from the Quilmes population (26°6'S, 65°9'W), where both sibling species are sympatric. This population and that of Arroyo Escobar (see above) are approximately 1150 km apart (see Hasson *et al.*, 1995; for information about the many differences between these populations). Previous work on this population showed that the pattern of morphometric differences between these species is equal in both sexes (Norry, 1995), particularly for the head traits examined here. The flies examined were the offspring of a random mating performed with the laboratory G2 generation of 73 (*D. koepferae*) or 48 (*D. buzzatii*) isofemale lines from wild inseminated females collected over banana bait buckets (species were identified by examining the male genitalia, see Vilela, 1983). The random mating was performed within each species by releasing three virgin flies of each sex and isofemale line into an egg-collecting chamber (100 × 200 × 300 mm). Samples of 30–40 first-instar larvae were collected daily from this chamber and transferred to each of 10 *standard* vials for optimal growth at 24 ± 1°C. For each species, 50 randomly chosen males (5 from each vial) emerging from these cultures (i.e. the laboratory G3 generation) were measured.

Estimation of genetic parameters and phenotypic correlations

Genetic parameters and their standard errors were estimated using the standard parametric procedures described in Falconer (1989). All data (in ocular units) were log_e-transformed and corrected to remove sexual dimorphism in the mean value of each trait. This correction was done by adding to male measurements the difference between female and male means.

Heritabilities were estimated by regressing mean values of offspring on mid-parent values (Falconer, 1989). This method assumes that the sexes do not significantly differ in phenotypic variance (Falconer, 1989), which was verified (Bartlett's test results for log_e-transformed data on the 630 individuals measured in this study are: $\chi^2 = 3.32$ for FW, $\chi^2 = 3.11$ for EW; $\chi^2 = 0.90$ for HW. In all cases, d.f. = 1; $P > 0.05$). In addition, we have no

evidence of additive X-linked variance in these traits in the same population (Norry *et al.*, 1997; present study).

Genetic correlations (r_g) were computed from midparent–offspring covariances (Falconer, 1989), obtaining two estimates for each pair of traits X and Y:

$$r_1 = \text{cov}(X'', Y') / [\text{cov}(X'', X') \text{cov}(Y'', Y')]^{1/2},$$

$$r_2 = \text{cov}(X', Y'') / [\text{cov}(X'', X') \text{cov}(Y'', Y')]^{1/2},$$

where '' refers to mid-parent and ' to offspring values. The average of r_1 and r_2 is the estimated genetic correlation. Standard errors of r_g were estimated using Reeve's (1955) formula:

$$\text{SE}(r_g) = [(1 - r_g^2) / \sqrt{2}] [\text{SE}(h_x^2) \text{SE}(h_y^2) / (h_x^2 h_y^2)]^{1/2}.$$

Phenotypic correlations were computed as the Pearson product–moment correlation between traits. Statistical significance of both genetic and phenotypic correlations was assessed for a matrix-wide P -value of 0.05, using the sequential Bonferroni test (Rice, 1989).

Precise estimation of genetic correlations between the sexes requires a half-sib design (e.g. Cowley & Atchley, 1988). Therefore, no such estimate was obtained.

Morphological divergence

Interspecific differences in each trait were estimated in males, using:

$$\text{DZ} = X_i - X_j,$$

where X_i and X_j are, respectively, the mean values (in mm) in *D. buzzatii* and *D. koepferae*. In this study, the analysis is arbitrary with respect to whether *D. koepferae* or *D. buzzatii* is the 'ancestral' species. In particular, we are concerned not with a retrospective selection analysis (Lande, 1979) but with the hypothesis that negatively correlated traits have evolved in opposite directions, which may be verified by examining whether the traits differ, in sign, for the DZ-values.

One-way ANOVA and Scheffe multiple comparisons for heterogeneity of means were performed for each trait. As the sample size of males of each species was 50 for the Quilmes population (see above), the analysis was balanced by taking a randomly chosen sample of 50 male parents of the heritability experiment for the Arroyo Escobar population.

Results

Genetic and phenotypic correlations between the traits

Heritability estimates were all significant, with moderate to low values for all traits (Table 1). The slopes of dam-offspring regressions were roughly similar to the slopes of sire-offspring regressions (results not shown), with no significant difference in an analysis of covariance of the slopes (the highest F -value was obtained for EW: $F_{1,206} = 2.50$, $P > 0.05$). Thus, there is no apparent evidence of additive X-linked variance (see Norry *et al.*, 1997; for similar results in other traits).

Genetic correlations were moderate in magnitude, with only one of the r_g -values being negative and significant at a matrix-wide P -value of 0.05 (Table 1). This negative r_g corresponds to the correlation between FW and EW (Table 1).

A phenotypic correlation pattern is evident in *D. buzzatii* from Arroyo Escobar, again with a negative value for the correlation between FW and EW, and positive values for the correlations of HW with both FW and EW (Table 1). This phenotypic correlation pattern is also observed in laboratory-reared males of both *D. koepferae* and *D. buzzatii* derived from the Quilmes population (Table 2).

Morphological divergence

Means and SD of the studied traits are shown in Table 3 for males, in both *D. buzzatii* and *D. koepferae*. Differences in trait means are significant between these

Table 1 Heritabilities (on diagonal), and genetic (below diagonal) and phenotypic (above diagonal) correlations among three head traits in laboratory-reared *D. buzzatii* derived from the Arroyo Escobar population. Phenotypic correlations are for parents and offspring combined (630 flies of the heritability experiment). Standard errors are in italics

	HW	FW	EW
HW	0.30** <i>0.09</i>	0.54***	0.62***
FW	0.35* <i>0.20</i>	0.26** <i>0.09</i>	-0.31**
EW	0.44* <i>0.21</i>	-0.48* <i>0.21</i>	0.22* <i>0.10</i>

Boldface correlations are significant at a matrix-wide P -value of 0.05 (sequential Bonferroni test). Individual P -values (i.e. without applying a Bonferroni technique) are: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$.

species, except for HW (Table 3). Because no significant difference was detected between *D. buzzatii* populations (Table 3), DZ-values are shown only for the between-species comparisons (Fig. 2). A divergence pattern is evident: FW has evolved in an opposite direction to EW (Fig. 2), with these traits exhibiting highly significant differences between species (Table 3). The apparent stability in HW is largely a by-product of this pattern of opposite changes in FW and EW, as DZ_{HW} -values are approximately equivalent to the sum of the respective DZ_{FW} and DZ_{EW} -values (Fig. 2).

Discussion

A negative genetic correlation was found between face width and eye width in laboratory-reared flies of *D. buzzatii* derived from the Arroyo Escobar population. Phenotypic correlations between these traits were also negative in laboratory-reared flies of *D. buzzatii*

Table 2 Phenotypic correlations among three head traits in laboratory-reared males of *D. buzzatii* (below diagonal) and *D. koepferae* (above diagonal) derived from the Quilmes population

	HW	FW	EW
HW		0.57***	0.74***
FW	0.54***		-0.31**
EW	0.71***	-0.20*	

Boldface values are significant at a matrix-wide P -value of 0.05 (sequential Bonferroni test). Individual P -values (i.e. without applying a Bonferroni technique) are: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$.

Table 3 Mean value (in mm) and SD (italics) for three developmentally related traits of the head capsule in laboratory-reared males of: *D. koepferae* from the Quilmes population (DKQ), *D. buzzatii* from the Quilmes population (DBQ), and *D. buzzatii* from the Arroyo Escobar population (DBAE). Results of ANOVA and Scheffé multiple comparisons for heterogeneity of means are also shown. Homogeneous means are connected by a continuous underline

Trait	DKQ	DBQ	DBAE	$F_{2,147}$
HW	<u>0.939</u> <i>0.027</i>	<u>0.940</u> <i>0.020</i>	<u>0.940</u> <i>0.026</i>	0.16
FW	0.420 <i>0.018</i>	<u>0.408</u> <i>0.015</i>	<u>0.408</u> <i>0.018</i>	5.46*
EW	0.516 <i>0.019</i>	<u>0.532</u> <i>0.015</i>	<u>0.530</u> <i>0.016</i>	8.57**

* $P = 0.005$; ** $P = 0.0004$.

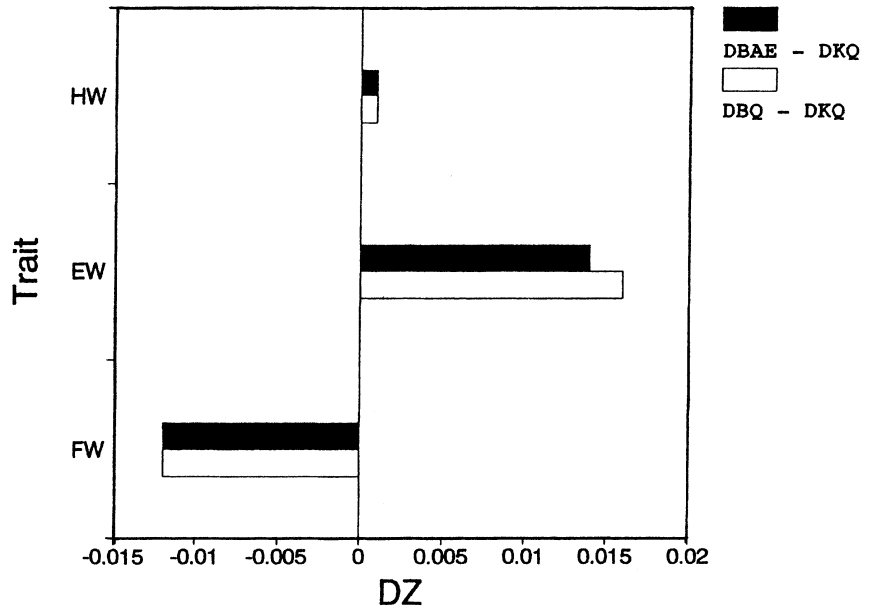


Fig. 2 Difference (DZ, in mm) in each trait between laboratory-reared males of *D. buzzatii* and *D. koepferae* derived from two natural populations: DBAE, *D. buzzatii* from the Arroyo Escobar population; DBQ, *D. buzzatii* from the Quilmes population; DKQ, *D. koepferae* from the Quilmes population. Abbreviations of traits are given in Fig. 1.

and *D. koepferae* derived from a different population (Tables 1 and 2). Similar results were obtained by David Cowley (discussed in Riska, 1986; p. 1307; see also Cowley & Atchley, 1990) in *D. melanogaster*. Thus, negative correlations between face width and eye width may be widespread in *Drosophila*.

This negative correlation is even more apparent in the pattern of morphological divergence between species. Head shape has diverged between *D. buzzatii* and *D. koepferae*. Specifically, FW and EW exhibit negatively correlated and highly significant DZ-values (Fig. 2), with HW showing no significant difference between populations/species. Thus, the two negatively correlated traits, FW and EW, have evolved in *opposite* directions in these two sibling species. The very consistent pattern of interspecific differentiation (Fig. 2) is supporting evidence of constraints on the range of likely combinations of mean traits. The overall picture of this divergence pattern is consistent with the present evidence for negative correlations between FW and EW. In addition, an apparent way for r_g -related constraints to emerge when, as is suggested for HW (Fig. 2), the apparently more constrained trait is the result (e.g. the sum) of two *negatively* correlated traits.

Because r_g s were estimated in *D. buzzatii* but not in *D. koepferae*, a possible difficulty in these comparisons is that genetic correlations among the traits could differ, in sign, between these sibling species. However, this possibility is unlikely, because the genetic correlation between face width and eye width is negative also in the less closely related *D. melanogaster* (Cowley & Atchley, 1990). Furthermore, it is remarkable that for our set of studied traits, the genetic correlations estimated in

D. buzzatii are similar, in sign, to their phenotypic counterparts, not only in *D. buzzatii* but also in both its sibling species *D. koepferae* (Tables 1 and 2) and the less closely related *D. melanogaster* (Cowley & Atchley, 1990). Thus, it is unlikely that the overall picture of negative correlations suggested by the results would be incorrect for our two sibling species. In addition, the negatively correlated traits, FW and EW, have evolved in opposite directions in many *Drosophila* species, including *D. melanogaster* and its sibling species *D. simulans* (results not shown).

Traits representative of overall size of head, thorax and wings tend to be positively correlated in *Drosophila*, at least in *D. melanogaster* (Cowley & Atchley, 1990) and *D. buzzatii* (Norry *et al.*, 1997). In addition, while functional (selective) relationships between traits may have substantial effects on r_g values (Sheridan & Barker, 1974), development is probably also a major determinant of pleiotropic relationships between morphological traits in *Drosophila* (Cowley & Atchley, 1990). The traits examined in the present study constitute an example of developmentally related traits, as these traits are derived from a common precursor, the *eye-antennal* discs (Morata & Lawrence, 1979). It is possible that negative correlations between face width and eye width reflect subdivision of the imaginal disc cells into a population that forms the multifaceted eye and a population that forms tissue that is not destined to form eye, as first suggested by Cowley & Atchley (1990). In fact, negative r_g values are predicted when the traits arise through a subdivision of a developmental precursor into variable parts (Riska, 1986), and negative correlations between face and eye widths

are probably widespread in *Drosophila* (Cowley & Atchley, 1990; present study). Alternatively, negative correlations may also be the result of competition between processes for a resource (Atchley, 1987) or functional relationships between the traits (but see Sheridan & Barker, 1974; for expected r_g values under two-trait selection with a resource competition model). The well-known case of *D. heteroneura* and *D. silvestris*, with the putative importance of behaviour in the topography of eyes for mate recognition (Templeton, 1977; but see Price & Wake, 1995), might suggest that some functional relationships are possible between the studied traits. While these traits are correlated with mating success in *D. buzzatii* (Norry *et al.*, 1995; 1996), they could be influenced either by developmental or functional effects, or both. Another interesting example of negative r_g that probably reflects developmental effects is the correlation between the proximal wing length and the distal wing length in *D. buzzatii* (Thomas & Barker, 1993; Loeschcke *et al.*, 1999).

Further observations are needed to test these alternative explanations. However, despite some uncertainty over the correlation's cause(s), our present observations do indicate that the two negatively correlated parts of the *Drosophila* head evolve typically in opposite directions, resulting in substantial interspecific divergence in head shape. For morphometric traits that are consistently negatively correlated as in the present study, their resulting patterns of interspecific divergence and shape variation could severely be biased in a trend that is consistent with the negative sign of r_g .

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