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Patterns of variation in wing morphology in the cactophilic *Drosophila buzzatii* and its sibling *D. koepferae*

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Abstract

Drosophila buzzatii and *D. koepferae* are two sibling species that breed on the necrotic tissues of several cactus species and show a certain degree of niche overlap. Also, they show differences in several life history traits, such as body size and developmental time, which probably evolved as a consequence of adaptation to different host plants. In this work we investigate the ecological and genetic factors affecting wing morphology variation both within and between species. Three wing traits were scored, distal and proximal wing length and width in isofemale lines reared in two of the most important host cacti: *Opuntia sulphurea* and *Trichocereus terschekii*. Our results revealed that differences between species and sexes in wing size and shape were significant, whereas the cactus factor was only significant for wing size. Intraspecific analyses showed that differences among isofemale lines were highly significant for both size and shape in both species, suggesting that an important fraction of variation in wing morphology has a genetic basis. Moreover, the line by cactus interaction, which can be interpreted as a genotype by environment interaction, also accounted for a significant proportion of variation. In summary, our study shows that wing size is phenotypically plastic and that populations of *D. buzzatii* and *D. koepferae* harbour substantial amounts of genetic variation for wing size and shape. Interspecific differences in wing size and shape are interpreted in terms of spatial predictability of the different host plants in nature.

Introduction

A central issue in evolutionary biology is to understand the mechanisms that promote morphological evolution, such as those responsible for size and shape variation in nature. In this context, any approach aimed to investigate morphological variation should include the genetic and environmental factors causing intraspecific variation and interspecific divergence (Mackay, 2004).

Body size and shape are heritable traits (e.g. Guerra *et al.*, 1997; Pezzoli *et al.*, 1997; Moraes *et al.*, 2004) that correlate with several fitness components (e.g. reviewed in Powell, 1997; Fernández Iriarte & Hasson, 2000; Cortese *et al.*, 2002). However, the relationship between body size and fitness with physiological and/or behavioural traits is quite complex (e.g. Schmidt-Nielsen, 1984). Moreover, size related traits, such as wing length, are known to be involved in a trade-off with developmental time (Fanara *et al.*, 1999; Fernández Iriarte & Hasson, 2000; Cortese *et al.*, 2002).

Insect wings, causally related to the spectacular diversification of insects, are a particularly attractive system for comparative studies (Carrol *et al.*, 1995; Gibson, 1999). Studies in natural populations of *Drosophila* have shown that the wing is a suitable model in studies of morphological evolution (e.g. Bitner-Mathé & Klaczko, 1999a,b; Huey *et al.*, 2000). Moreover, it is well known that wing size and shape respond to environmental variation in complex ways, suggesting that the reaction norm may be part of an adaptive response (e.g. Weber,

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1990; Thomas & Barker, 1993; Bitner-Mathé & Klaczko, 1999b). The plot of mean phenotypic values of a genotype across a range of environments is a possible way of visualizing its reaction norm, or environmental sensitivity; whereas phenotypic plasticity refers to the property of a genotype to produce different phenotypes in response to an environmental change (e.g. Via *et al.*, 1995; Schlichting & Pigliucci, 1998; Conner & Hartl, 2004). In addition, the response to changes in environmental conditions may vary among genotypes (Gomulkiewicz & Kirkpatrick, 1992; Gavrilets & Scheiner, 1993). Such variation in reaction norms among genotypes may be evidenced as a genotype by environment interaction ($G \times E$) (Falconer, 1990; Lynch & Walsh, 1998). If none of the genotypes has the maximum fitness across all environments, selection may favour alternative genotypes in different environmental patches (Thomas & Barker, 1993; Ungerer *et al.*, 2003). Abundant experimental and theoretical work gives strong support to the idea that $G \times E$ may be involved in the maintenance of phenotypic plasticity, genetic variation and the evolution of fitness related traits (Fernández Iriarte & Hasson, 2000; Ungerer *et al.*, 2003; Fanara *et al.*, 2006). However, most of these studies have been performed in species whose ecology is poorly known, preventing the possibility of simulating appropriate ecological conditions in the laboratory and extrapolations to natural conditions.

Cactophilic species of the genus *Drosophila* are ideal models in studies of life history evolution, as most species are adapted to the use of diverse host plants that may potentially represent contrasting environments for the flies (Hasson *et al.*, 1995; Powell, 1997; Fanara *et al.*, 1999). *Drosophila buzzatii* and *D. koepferae* are two closely related species of the *D. buzzatii* complex (*repleta* group, *mulleri* subgroup, Ruiz & Wasserman, 1993), that have partially overlapping distributions in the arid lands of Northwestern and Western Argentina (Fontdevila *et al.*, 1988; Fanara *et al.*, 1999). Both species can utilize the necroses of several species of the genus *Opuntia* (prickly pears or tunas) and columnar cacti of the genera *Cereus* and *Trichocereus* as breeding substrates (Hasson *et al.*, 1992). However, these species show a certain degree of niche specificity. In fact, surveys in natural populations have shown that, 81% of *D. buzzatii* flies emerge from tunas and secondarily (19%) from columnars (cardón), whereas the reverse is true for *D. koepferae* (7% and 93% from tunas and columnars, respectively; Hasson *et al.*, 1992). Nevertheless, flies of the same species emerging from tunas and columnars do not show any sign of genetic isolation (Hasson *et al.*, 1992).

Although *D. buzzatii* and *D. koepferae* are undistinguishable by their external morphology, the former has a smaller body size and develops faster than the latter. Such divergence has supposedly occurred as the outcome of adaptive evolution in association with the use of resources that differ in duration, abundance and dispersion (Fanara *et al.*, 1999; Fanara & Hasson, 2001; see also Etges, 1990 for a comparable picture in *D. mojavensis* and allied species). Tunas are considered a more ephemeral breeding substrate but more abundant as a feeding site than columnars, and those species that use tunas tend to be smaller and develop faster (Etges, 1990; Fanara *et al.*, 1999). Thus, the larger body size reported for *D. koepferae* may be a consequence of natural selection for greater dispersal ability given the greater average distance between breeding sites than in the case of opuntia feeders like *D. buzzatii* (Etges, 1990; Fanara *et al.*, 1999).

Although dispersal ability appears to have played a pivotal role, either as a cause or as a consequence of adaptation to different hosts in these species, studies aimed at investigating wing morphology in *D. koepferae* and *D. buzzatii* are lacking. In this paper we investigate the ecological and genetic components of intra and interspecific variation in wing morphology (size and shape). The present effort is part of an ongoing project directed at investigating the ecological and genetic basis of interspecific phenotypic differences and the role of phenotypic plasticity and $G \times E$ in the evolution of host plant use in these pair of sibling species inhabiting South American deserts.

Materials and methods

Collection and maintenance of stocks

Strains of *D. buzzatii* and *D. koepferae* were founded with flies collected in the locality of Suyuque (33.3°S, 66.5°W, San Luis Province, Argentina) where both species coexist in almost equal proportions. The rotting cladodes of *Opuntia sulphurea* and the stems of *Trichocereus terschekii* are the main, although not the only, potential breeding and feeding resources for both species in this locality.

Flies were collected by net sweeping on fermented banana baits in late summer 2003. Captured flies were sexed in the laboratory and isofemale lines were set up by placing single wild-caught females in vials containing 5 mL of David's killed yeast culture medium (David, 1962). Each line was identified to species by the inspection of the genitalia of one male progeny (Vilela, 1983), as females of both species are morphologically indistinguishable. Isofemale lines of *D. koepferae* and *D. buzzatii* were reared under identical conditions in vials with 5 mL of laboratory medium for five generations before the onset of the experiments.

We also collected rotting cladodes and fresh material of both *O. sulphurea* and *T. terschekii* in the same locality for the preparation of two types of 'semi-natural' media. Pieces of fresh cacti were stored at −20 °C and the fermenting juices exudated from several cactus necroses of both species were maintained in the laboratory by adding 10 g of fresh cactus every 2 weeks. For the preparation of the cactus media, pieces of fresh cactus of each species were mixed in a blender and 10 mL poured into standard glass vials (2.5 × 12 cm). Vials were then

autoclaved and after cooling, each one was inoculated with 0.1 mL of the fermenting juice obtained from naturally occurring rotting materials of the corresponding cactus species in order to start the fermentation process.

Experimental design

Fifteen isofemale lines of each species were randomly chosen from the original set. For each line, 100 pairs of sexually mature flies were placed in egg-collection chambers (two chambers per isofemale line) as described in Fanara *et al.* (1999). Eggs were allowed to hatch and batches of 30 first-instar larvae were transferred to culture vials containing a 'semi-natural' medium prepared with fermented *O. sulphurea* or *T. terschekii*. Four replicated vials were run for each combination of cactus and isofemale line. Larvae were raised at 25 ± 1 °C with 12 : 12 light/dark until the emergence of adult flies.

Three to five flies of each sex emerged in each vial were randomly chosen and both wings of each individual were removed and mounted on microscope slides. Wing images were captured using a binocular microscope (10×) and an attached digital camera connected to a computer. For each wing we scored the following traits: proximal wing length (WLp), distal wing length (WLd) and total width (WW; Fig. 1), using TPS DIG (Rohlf, 2001, v. 1.31, available at http://life.bio.sunysb.edu/morph/).

Statistical analysis

As the different wing traits scored are highly correlated, we decided to perform a principal component analysis (PCA) using the average of both wings (in mm × 100) for all traits. PCA allows to generate a new set of independent variables, the principal components (PCs), from the original variables. The scores for the new variables for each fly were employed in the ANOVAs described below.

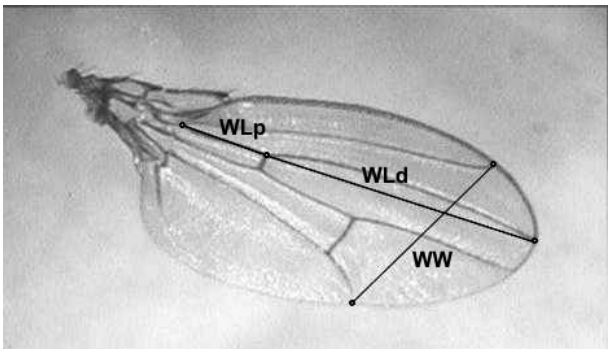

Fig. 1 A representative image of the wing of *Drosophila buzzatii* and *D. koepferae* showing the size traits scored in each fly. WLp, proximal wing length; WLd, distal wing length; WW, wing width.

General analysis

Variance of the first two PCs was partitioned into sources of variation attributable to *Drosophila* species, Cactus and Sex, using the following ANOVA fixed model:

$$Y = \mu + E + S + C + (E \times S) + (E \times C) + (S \times C) + (E \times S \times C) + \epsilon$$

where μ is the overall mean of the trait, E, S and C stand for the effects of species, sex and cactus respectively, and ϵ represents the error. In turn, interactions involving the species factor allow to test whether *D. buzzatii* and *D. koepferae* respond differentially to variation in breeding conditions (differences between flies reared in different cacti) or to the sexual environment (differences between sexes).

Within species analyses

Additional ANOVAs for each combination of species and sex were conducted to investigate the sources of phenotypic variation within each species for both PC1 and PC2, according to the model:

$$Y = \mu + L + C + (L \times C) + R(L \times C) + \epsilon$$

where μ is the overall mean, C is the cactus effect (fixed), L stands for the line effect (random), R is the replicate effect (random, nested within the $L \times C$ interaction) which tests for differences among replicates, and ϵ represents the error.

A significant line effect can be interpreted as because of genetic differences among lines, whereas a significant cactus effect as phenotypic plasticity (Debat & David, 2001; Conner & Hartl, 2004; David *et al.*, 2005). Finally, a significant $L \times C$ interaction may be interpreted as an estimation of the genotype by environment interaction ($G \times E$).

A significant $G \times E$ can arise as a consequence of differences in among-lines variance in separate environments (change in scale) and/or deviations from unity of the cross-environment genetic correlation ($rG \times E < 1$; see below) (change in ranking order). The contribution of the two sources of variation to $G \times E$ was analysed by means of the equation derived by Robertson (1959):

$$V_{G \times E} = [(\sigma_{E1} - \sigma_{E2})^2 + 2\sigma_{E1}\sigma_{E2}(1 - rG \times E)]/2$$

where $V_{G \times E}$ is the $G \times E$ variance component, $rG \times E$ is the cross-environment genetic correlation and, σ_{E1} and σ_{E2} are the square roots of the among-line variance components in the two environments (*O. sulphurea* and *T. terschekii*). σ_{E1} and σ_{E2} were obtained from the ANOVAs performed for each combination of species and cactus host and represent the square roots of the among lines variance component in each host plant. The first term corresponds to differences in among-line variance whereas the second to deviations from the perfect correlation across environments ($rG \times E < 1$).

The cross-environment genetic correlation ($rG \times E < 1$) is the genetic correlation of measurements

of the same trait in different environments and here reflects the degree in which the same genes control trait expression across cactus hosts. $rG \times E$ was estimated for each trait as:

$$rG \times E = \mathrm{COV}_{\mathrm{E1\,E2}}/\sigma_{\mathrm{E1}}\sigma_{\mathrm{E2}}$$

where $\mathrm{COV}_{\mathrm{E1\ E2}}$ is the covariance of lines means for each sex measured in different cactus hosts. We estimate the $\mathrm{COV}_{\mathrm{E1\ E2}}$ as the covariance of line means in the different environments. This method for the estimation of $rG \times E$ is not equivalent to the computation of a product-moment correlation or the genetic correlation across environments (Lynch & Walsh, 1998 p. 663).

All statistical analyses were performed using GLM procedure implemented in the STATISTICA 6.0 software package (StatSoft, Inc, 2001). In all analyses the Bonferroni correction for multiple tests was applied.

Results

Mean values and standard deviations of WLd, WLp and WW in males and females of both species reared in both cactus hosts are given in the Table A1. All three morphological traits were scored in 1602 flies wherein, on average, *D. koepferae*, females and flies reared in *O. sulphurea* were larger than *D. buzzatii*, males and flies reared in *T. terschekii*, respectively.

Two principal components, PC1 and PC2, accounting for more than 95% of variation (Table 1) were extracted from the correlation matrix. All original variables loaded heavily and negatively on PC1 (Table 1). Thus, PC1, which alone accounted for 77% of variation, may be taken as a good indicator of variation in general size. Particularly, high scores of PC1, which is negatively correlated to WW, WLp and WLd, indicate small values of the three wing traits and vice versa. One of the traits (WLd) loaded negatively, whereas the other two positively on PC2, which explained nearly 19% of variation (Table 1). Hence, PC2 can be considered as a measure of wing shape; low PC2 scores indicate wings with long distal veins (WLd), short proximal veins (WLp) and narrow wings (WW) and vice versa.

Table 1 Loading of the three linear measurements of wings: proximal wing length (WLp), distal wing length (WLd) and total width (WW) of *Drosophila koepferae* and *D. buzzatii* on the three principal component axes and the variance explained for each one of them.

	PC 1	PC 2	PC 3
Factor loadings			
WLp	−0.912	0.325	0.249
WLd	−0.766	−0.641	0.038
WW	−0.939	0.208	−0.273
Explained variance (%)	76.748	18.657	4.594

Linear measurements correspond to those in Fig. 1.

Table 2 ANOVAS of PC1 and PC2 pooled across species (*Drosophila koepferae* and *D. buzzatii*), cacti (*Opuntia sulphurea* and *Trichocereus terschekii*) and sexes (male and female).

		PC 1		PC 2	
Source of variation	d.f.	MS	*F*-value	MS	*F*-value
Species	1	1285.1	1259.3**	57.0	110.9**
Cactus	1	180.2	176.6**	0.1	0.2
Sex	1	581.7	570.0**	15.3	29.7**
Species × cactus	1	2.8	2.7	2.3	4.4
Species × sex	1	13.4	13.1*	1.1	2.1
Cactus × sex	1	3.0	3.0	0.3	0.6
Species × cactus × sex	1	2.8	2.8	0.4	0.8
Error	1594	1.0		0.5	

*$P < 0.005$ (maximum signification level after correction for multiple tests).
**$P < 0.0001$.

General analysis

The PC1 differences between species, between flies reared in different cacti and between sexes were highly significant (Table 2). *Drosophila koepferae* had smaller scores of PC1 than *D. buzzatii*. Similarly, females and flies emerged from *O. sulphurea* had, on average, smaller scores of PC1 than males and flies grown in *T. terschekii* vials, respectively. The species by sex interaction was also significant and it can be accounted for by the greater differences in size between sexes in *D. buzzatii* than in *D. koepferae*.

Analysis of the second principal component (PC2) revealed significant differences between species and sexes. *Drosophila koepferae* flies and males presented, on average, higher scores for PC2 than *D. buzzatii* and females, respectively, indicating that the formers (*D. koepferae* and males) tended to have shorter distal wing veins, longer proximal veins and wider wings than *D. buzzatii* and females.

Within species analysis

A summary of the results of the partial ANOVAS, aimed to investigate the relative contribution of the genetic component of variance (differences among isofemale lines) and the effect of cactus hosts to phenotypic variance in each sex and species separately, are presented in Table 3.

For PC1, both main sources of variation, namely cactus and line, were significant in males and females of both species. The line by cactus interaction ($G \times E$) was also highly significant and accounted for a substantial amount of variance (15.2 – 26%). In all cases, the percentage of variance explained by the $G \times E$ was invariably greater than the line factor.

These results prompted us to perform additional ANOVAS to evaluate the significance and the relative contribution of the line factor to total variance, in each

Table 3 ANOVA results and quantitative genetic statistics of plasticity and $G \times E$ for PC1 and PC2 of males and females of *Drosophila buzzatii* and *D. koepferae* measured in *Opuntia sulphurea* and *Trichocereus terschekii* cactus hosts. Percentage of variances explained for each factor is expressed between brackets.

	Cactus†	$[V_L]$‡	$[V_{L \times C}]$§	$[V_{L(O.s.)}]$¶	$[V_{L(T.t.)}]$††	$[r_{(G \times E)}]$‡‡	$[V_{L \times C}]$ partitioned§§
D. buzzatii							
Males							
PC1	**	0.095 (11.7)**	0.137 (16.9)**	0.223 (31.3)**	0.247 (27.0)**	0.358	[0.1–99.9]
PC 2	NS	NS	NS	NA	NA	NA	NA
Females							
PC1	**	0.039 (4.1)**	0.144 (15.2)**	0.159 (21.6)**	0.201 (17.2)**	0.171	[0.7–99.3]
PC 2	NS	0.021 (5.7)*	NS	NA	NA	NA	NA
D. koepferae							
Males							
PC1	**	0.000 (0.0)**	0.347 (26.0)**	0.302 (23.5)**	0.153 (13.5)**	–0.628	[5.5–94.5]
PC 2	NS	0.050 (10.2)**	NS	NA	NA	NA	NA
Females							
PC1	**	0.091 (7.5)**	0.292 (24.1)**	0.226 (23.3)**	0.517 (36.3)**	0.247	[8.0–92.0]
PC 2	NS	0.069 (9.8)**	NS	NA	NA	NA	NA

*$P < 0.005$; **$P < 0.0001$; NA, not applicable; NS, not significant.

†Fixed effects of host cacti from each principal component ANOVA model; significance indicates plasticity for cacti (*O. sulphurea* and *T. terschekii*).

‡Among-isofemale lines variance component from each principal component ANOVA model, significance denotes genetic differences among isofemale lines reared in both cacti.

§Line by cactus interaction variance component from each principal component ANOVA model; significance indicates genotype × environment interaction ($G \times E$).

¶Among-isofemale lines variance component from each principal component ANOVA model; significance indicates genetic differences among isofemale lines reared in *O. sulphurea*.

††Among-isofemale lines variance component from each principal component ANOVA model; significance indicates genetic differences among isofemale lines reared in *T. terschekii*.

‡‡Cross-environment genetic correlation calculated as $\mathrm{COV}_{\mathrm{E1\ E2}}/\sigma_{\mathrm{E1}}\sigma_{\mathrm{E2}}$.

§§Proportions of $V_{G \times L}$ attributable to changes in among-line variance in both host cacti environments (first term) and to the departure of the cross-environment genetic correlation from unity (second term).

combination of species, cactus and sex. Differences among isofemale lines were highly significant and explained a substantial amount of total phenotypic variance, from 13.5% to 36.3% in *D. koepferae* and from 17.2% to 31.3% in *D. buzzatii* (Table 3).

In the case of PC2, only differences among isofemale lines were significant except in *D. buzzatii* males. Neither the cactus nor the line × cactus interaction were significant in the ANOVAs performed separately for *D. buzzatii* and *D. koepferae*.

We also investigated the relative contribution of differences in among-lines variance in separate environments (a change in scale) and/or deviations of the cross-environment genetic correlation from unity (a change in rank order) to the significant $G \times E$ (Table 3). The results of these tests showed that the significance of the $G \times E$ was mainly due, in all cases, to changes in the ranking order (varied from 92.0% to 99.9% and the change in scale varied from 0.1% to 8.0%), i.e. large-winged lines in *O. sulphurea* tended to have shorter wings when reared in *T. terschekii* and vice versa.

Discussion

The main findings in the present study can be summarized in the following points: (i) flies emerged in the two cacti differ in wing size, (ii) *D. buzzatii* and *D. koepferae* harbour considerable amounts of genetic variation, mainly for wing size, and also for wing shape, (iii) size variation among isofemale lines (within species) was not independent of the cactus host and (iv) interspecific differences in wing size and shape have evolved since these species started to diverge.

One important point arising from our study is that the factors affecting wing size and shape are different. For instance, the effect of the cactus host, which under our experimental design can be considered as an estimate of phenotypic plasticity, affected wing size but not shape. These results imply that different phenotypes arose in response to breeding in different cacti, in line with observations for other life history traits such as viability, developmental time and oviposition preference (Fanara *et al.*, 1999, 2004; Fanara & Hasson, 2001).

The second relevant issue is that differences among isofemale lines (within species) and the line by cactus interaction, which can be considered as estimates of the genetic component of variance and the genotype by environment interaction, respectively, accounted for an important proportion of wing size variation in *D. buzzatii* and *D. koepferae*. Again, it is remarkable that similar results were observed for viability and developmental time in another set of populations (Fanara *et al.*, 2006).

These results not only indicate that substantial genetic variance underlies wing size variation in both species, but also that genotypes responded differentially to breeding conditions. Thus, it seems reasonable to conclude that breeding on different cacti might impose varying selective pressures on extant genetic variation, which under certain conditions may lead to the maintenance of genetic variation. In this sense, a lavish literature documents that temporal and/or spatial variation in selection can contribute to the maintenance of genetic variation by $G \times E$ (Via & Lande, 1985; Gillespie & Turelli, 1989; Rodríguez *et al.*, 1999; Fernández Iriarte & Hasson, 2000; Ungerer *et al.*, 2003; Hedrick, 2005), suggesting the potential for sympatric diversification associated to host use in this pair of sibling species and in other cactophilic species of the repleta group (e.g. Heed & Mangan, 1986).

Several features differentiating tunas and columnars can be invoked to account for the fact that these two types of host cacti are perceived as different patches of a heterogeneous environment by these flies. First, prickly pears and columnar cacti differ, as breeding sites for cactophilic *Drosophila*, in several biologically relevant aspects such as chemical composition and diversity in the microflora (yeasts and bacteria) associated with the rotting process (Starmer *et al.*, 1990) as it was reported for the *Drosophila*-cactus-yeast model system in *Drosophila* species inhabiting the Sonoran desert (Fogleman & Abril, 1990). Secondly, there is evidence that columnar cacti are chemically more complex (Fogleman & Abril, 1990), although less nutritious for the flies than *Opuntia*, thus favouring more specialized, less plastic, flies. Thirdly, and more specifically, *O. sulphurea* and *T. terschekii* differ in rot size, larval density in the rots and rot duration in North Western Argentina (Hasson *et al.*, 1992).

Regarding wing shape, our results indicate that the main sources of variation are different from those detected for wing size. In fact, only the line factor contributed significantly to phenotypic variance; neither the cactus effect nor the $G \times E$ were significant, suggesting that shape is under stronger developmental constraints than size (Weber, 1990; Gilchrist & Partridge, 2001). In this context, studies of wing morphology have shown that the major compartments of the wing may behave as independent units of selection contributing differentially to wing size adaptation, as in *D. subobscura* (Huey *et al.*, 2000). Our results are in line with studies of wing development and morphology, in that different parts of the wing appear to vary in a concerted manner, as a kind of accommodation of different wing compartments to variation in another during development (Guerra *et al.*, 1997; Pezzoli *et al.*, 1997).

Finally, between species differences in wing size and shape were also significant. *D. koepferae* had larger wings than *D. buzzatii*, which is consistent with previous studies on thorax length (Fanara *et al.*, 1999). Such differences in general body size have been explained as an adaptive evolutionary response to the utilization of host plants characterized by differences in spatial and temporal predictability (Fanara *et al.*, 1999).

Studies in *Drosophila* have shown that body size (Roff, 1977; Thomas & Barker, 1993; Nunney & Cheung, 1997), wing shape (Cavicchi *et al.*, 1991; Bitner-Mathé & Klaczko, 1999a) and the ratio body size to wing length, wing loading, (Stalker, 1980) are related to dispersal ability. Moreover, several studies have shown that wing loading is related to flight cost and is probably an important target of selection (Loeschcke *et al.*, 1999). In this study we have only measured wing traits and observed that *D. koepferae* tended to have wings, which are 11% longer than *D. buzzatii*. Although we did not measured thorax length in the flies analysed in this paper, previous studies have shown that *D. koepferae* has a larger thorax length than *D. buzzatii* in flies reared in identical conditions than in the present report (Fanara *et al.*, 1999). However, between species differences for this trait were less pronounced than for wing length. Using our estimates of total wing length (WLp + WLd) and the values reported for thorax length, we estimated wing loading. This compound trait, which is inversely related to dispersal ability, is lower in *D. koepferae* than in *D. buzzatii*. Thus, differences between species in general body size and wing loading are in agreement with the idea that *D. koepferae*, which in nature is associated to less predictable (widely dispersed) resources like the columnar cactus *T. terschekii*, has a greater dispersal ability than *D. buzzatii*, which in nature is mainly associated to prickly pears, an abundant and spatially predictable resource (Hasson *et al.*, 1992; Fanara *et al.*, 1999). Moreover, the present study is in agreement with the idea that species utilizing spatially predictable patches have lower dispersal rates than species adapted to the use of less predictable or more widely dispersed resources (Fanara *et al.*, 1999; Markow & Castrezana, 2000).

Interestingly, a recent study has also tested this hypothesis by assessing dispersal rates in a guild of cactophilic species inhabiting the Sonoran desert (Markow & Castrezana, 2000). The authors' main conclusion is that *D. nigrospiracula*, the largest species in the guild, had the highest dispersal rate, as compared for instance to *D. pachea*. Furthermore, these results give support to the idea that differences in dispersal abilities are related to ecological features of each species' resources, in agreement with our prediction (Fanara *et al.*, 1999). In effect, *D. pachea* utilizes the rotting pockets of senita (*Lophocereus schotii*) cactus (Heed & Mangan, 1986) which are much denser and more frequently encountered than cardón (*Pachycereus pringlei*) and saguaro (*Carnegiea gigantea*), the cactus hosts of *D. nigrospiracula*. Although direct estimates of dispersal rates are necessary in *D. koepferae* and *D. buzzatii*, the present study provides a testable working hypothesis concerning the relationship between dispersal ability and the evolution of host plant use in cactophilic *Drosophila* inhabiting South American deserts.

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Appendix

Table A1 Mean values and standard deviations (SD) of proximal wing length (WLp), distal wing length (WLd) and total width (WW, in mm) in males and females of *Drosophila buzzatii* and *D. koepferae* flies reared in *Opuntia sulphurea* and *Trichocereus terschekii*.

	Males [mean (SD)]				Females [mean (SD)]			
	WLd	WLp	WW	*n*	WLd	WLp	WW	*n*
D. buzzatii								
O. sulphurea	1.450 (0.004)	0.412 (0.003)	1.002 (0.003)	195	1.540 (0.003)	0.448 (0.002)	1.055 (0.003)	215
T. terschekii	1.394 (0.004)	0.397 (0.003)	0.970 (0.003)	197	1.483 (0.005)	0.433 (0.002)	1.024 (0.004)	191
D. koepferae								
O. sulphurea	1.583 (0.006)	0.437 (0.002)	1.091 (0.005)	192	1.649 (0.005)	0.475 (0.003)	1.135 (0.004)	203
T. terschekii	1.558 (0.006)	0.427 (0.002)	1. 072 (0.004)	204	1.609 (0.006)	0.453 (0.003)	1.103 (0.004)	205

n = sample sizes.