

# Chromosomal inversions effect body size and shape in different breeding resources in *Drosophila buzzatii*

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The cactophilic *Drosophila buzzatii* provides an excellent model for the study of reaction norms across discrete environments because it breeds on rotting tissues (rots) of very different cactus species. Here we test the possible effects of second chromosome inversions on body size and shape (wing loading) across suitable natural breeding substrates. Using homokaryotypic stocks derived from several lines homozygous for four naturally occurring chromosomal inversions, we show that arrangements significantly affect size-related traits and wing loading. In

addition, karyotypes show differing effects, across natural breeding resources, for wing loading. The 2st and 2jz<sup>3</sup> arrangements decrease and the 2j arrangement increases wing loading. For thorax length and wing loading, karyotypic correlations across host plants are slightly lower in females than in males. These results support the hypothesis that these traits have a genetic basis associated with the inversion polymorphism.

*Heredity* (2003) 91, 51–59. doi:10.1038/sj.hdy.6800280

**Keywords:** chromosomal inversion; *Drosophila*; body size; wing loading; cactus hosts

## Introduction

Several studies have shown that inversion polymorphisms in *Drosophila* affect size-related traits (Ruiz *et al*, 1991; Hasson *et al*, 1992a) and body shape (Bitner-Mathé *et al*, 1995). The main interest in the linking of these chromosomal polymorphisms to phenotypic variation is that this may provide a mechanistic approach for investigating the causes of natural selection acting on these widespread polymorphisms.

There is an important body of evidence showing that body size is correlated with fecundity (Robertson, 1957), dispersal ability (Roff, 1977), mating success (Partridge *et al*, 1987; Santos *et al*, 1988), longevity (Santos *et al*, 1992; Hasson *et al*, 1993; Rodríguez *et al*, 1999; Reeve *et al*, 2000; Norry and Loeschke, 2002) and development time (Partridge and Fowler, 1993; Betrán *et al*, 1998; Fernández Iriarte and Hasson, 2000). Other studies have shown that body size (Thomas and Barker, 1993; Nunney and Cheung, 1997), wing shape (Cavicchi *et al*, 1991; Bitner-Mathé and Klaczko, 1999) and a composite trait related to flight ability, wing loading (Stalker, 1980), respond to environmental variation in complex ways (Loeschke *et al*, 1999), suggesting that reaction norms in these traits may be part of an adaptive exophenotypic response in *Drosophila*.

The cactophilic species *Drosophila buzzatii* provides an excellent model for the study of reaction norms across discrete environments because it breeds on rotting tissues (rots) of very different cactus species that coexist within a given locality (Hasson *et al*, 1992b). Rots of different cactus species may differ in yeast diversity (Starmer, 1981), chemical composition (Kircher, 1982) and other ecologically relevant variables such as size and relative density (Hasson *et al*, 1992b). Likewise, adaptive strategies in cactophilic *Drosophila* may be related to the temporal and spatial predictability of different cactus species (Etges, 1993; Fanara and Hasson, 2001). Given the potential importance of the maintenance of the genetic variation in heterogeneous environments, it is of considerable interest to test whether or not particular genotypes respond differentially to these discrete environments.

Inversion polymorphisms in *D. buzzatii* vary clinally along latitudinal and altitudinal gradients that are correlated with geographic variation in temperature (Hasson *et al*, 1995; Rodríguez *et al*, 2000). Moreover, second chromosome inversions in this species have been shown to affect thorax length (Ruiz *et al*, 1991; Hasson *et al*, 1992a; Norry *et al*, 1995b), longevity (Hasson *et al*, 1991; Rodríguez *et al*, 1999), developmental time and preadult viability (Betrán *et al*, 1998; Fernández Iriarte and Hasson, 2000). More surprisingly, the effects of polymorphic inversions on the latter two traits strongly depend on the cactus species where flies develop from egg to adult (Fernández Iriarte and Hasson, 2000). Here, we test the possible effects of second chromosome

inversions on body size and shape across different natural breeding resources.

## Materials and methods

Flies were collected in the natural population of Otamendi (Buenos Aires Province), where *D. buzzatii* breeds and feeds on the necrotic cladodes of *Opuntia vulgaris* (see Hasson *et al* (1995) for a detailed description of the site of collection). This population is polymorphic for four second chromosome inversions namely: st (0.157), j (0.697),  $jz^3$  (0.142) and  $jq^7$  (0.004) (number of chromosomes analyzed: 816).

After six to 10 generations of sib-mating, three homozygous lines of each arrangement were used to set up four homokaryotypic stocks (see below), in order to avoid differing levels of consanguinity among stocks. Four experimental stocks homozygous for each arrangement (st/st, j/j,  $jz^3/jz^3$  and  $jq^7/jq^7$ ) were established one generation before the experiment (see details in Fernández Iriarte and Hasson, 2000). A total of 100 5- to 6-day-old virgin flies of each sex and desired karyotype were placed in egg-collecting chambers in order to derive all 10 possible karyotypes (st/st, st/j, st/ $jz^3$ , st/ $jq^7$ , j/j, j/ $jz^3$ , j/ $jq^7$ ,  $jz^3/jz^3$ ,  $jz^3/jq^7$  and  $jq^7/jq^7$ ). Flies were allowed to mate and oviposit for 48 h in plastic boxes containing Petri dishes with an egg laying medium (15 g agar, 75 ml ethanol (95%), 15 ml glacial acetic acid in 1500 ml water). Dehydrated Baker's yeast was used to stimulate oviposition. Large numbers of eggs were collected and then sterilized with 50% clorox for 3 min (Starmer and Gilbert, 1982). Eggs were allowed to hatch, and sets of 40 axenic first-instar larvae were transferred to culture vials containing 6 g of three different types of cactus media, prepared with fresh tissues of *O. vulgaris* (Ov), *O. ficus-indica* (Of) or *T. terscheckii* (Tt). Ov is the host plant in the population from which the flies were derived and constitutes a suboptimal resource (Fernández Iriarte *et al*, 1999). Of is the main host plant in colonized populations of the Old World (Ruiz *et al*, 1986) and in some Argentinian populations (Fanara *et al*, 1996) and its higher amount of carbohydrates could positively affect adult fitness (Brazner *et al*, 1984). Although *D. buzzatii* is mainly an *Opuntia* breeder, it can also breed on necrotic stems of various species of columnar cacti of the genera *Trichocereus* and *Cereus* in North Western Argentina (Hasson *et al*, 1992b). Thus, we also included cardón, *Trichocereus terscheckii* (Tt), as a representative of columnar cacti which, in general, are thought to have a more complex chemical composition that may affect the microflora (yeasts and bacteria) associated with the necrotic pads (Starmer, 1981; Kircher, 1982). *D. buzzatii* use the rotting stems of *T. terscheckii* as breeding and feeding sites in nature (Hasson *et al*, 1992b). Estimates of viability of *D. buzzatii* flies in media prepared with rotting tissues of cardón (93%) were similar to those obtained in Of and Ov (90 and 89%, respectively) (Fernández Iriarte and Hasson, 2000). Therefore, Tt was considered as a suitable cactus host for *D. buzzatii*.

Pieces of cactus were ground into a blender and 6 g poured into standard vials (2.5 × 12 cm). Vials were then autoclaved and after cooling each one was inoculated with 0.1 ml of fermenting juice obtained from naturally occurring rots of the corresponding cactus species. The

fermenting juice of each cactus was obtained from several natural rots maintained by adding 10 g of fresh cactus every week. In total, 40 first-instar larvae were transferred to vials 24 h after the addition of the fermenting juice. Five replicated vials were set up per cactus medium and karyotype. Heterokaryotypic experimental individuals were obtained from both reciprocal crosses in equal proportions. Egg collecting chambers and vials were incubated at 25°C.

For each karyotypic class, 25 males and 25 females reared in each cactus medium (five flies of each sex from each replicate vial) were scored for the following size-related traits: thorax length (TL), wing length (WL), wing width (WW), head width (HW), and face width (FW) (see Norry *et al* (1995a) for details). We also analyzed, as an estimate of shape, the thorax/wing length ratio (TL/WL) which is directly related to the wing loading (but see Thomas and Barker, 1993; Loeschcke *et al*, 1999). All measurements were performed with a Wild microscope fitted with an ocular micrometer.

## Statistical analysis

All traits were  $\log_{(e)}$ -transformed prior to analyses. ANOVAs were performed separately for each sex and trait, using karyotype and cactus species as fixed factors and replicate (vial) as a random factor nested within each fixed factor.

Genetic variability in plasticity was evaluated first in terms of genotype-by-environment ( $G \times E$ ) interaction (ie, karyotype × cactus interaction) by plotting mean values of the second chromosome karyotypes across cactus hosts. Second, we estimated the correlation between pairs of traits in different environments (Falconer and Mackay, 1996). Karyotypic correlations (Spearman rank) and confidence interval (Zar, 1984) were estimated by using the mean value of each karyotype for each trait in each cactus medium (Fernández Iriarte and Hasson, 2000). The first method provides a test of magnitude of  $G \times E$  interaction and the last method provides information on the direction of the interaction. A correlation of 0.8 or less indicates a substantial difference in the direction of response with 'biological significance' (Robertson, 1959).

The linear contribution of each inversion arrangement to the phenotypic value of each trait was tested in each breeding substrate by means of linear regressions on chromosome dosage (Ruiz *et al*, 1991). For each arrangement we consider the following classes (or chromosome dosage): homozygotes (chromosome dosage 2), heterozygous individuals carrying the gene order under consideration (chromosome dosage 1) and individuals not carrying the arrangement under consideration (chromosome dosage 0). This procedure allows the partition of total phenotypic variance into two components of karyotypic variance: additive, given by the proportion of variance explained by the regression ( $R^2$ ), and nonadditive (dominance plus epistasis), given by the proportion of variation not explained by linear regression.

Since chromosome dosage for a given arrangement is a linear combination of the remaining ones we used the all-subsets methods to find the best reduced model, the one with the largest  $R^2$  coefficient in the multiple regression.

**Table 1** Two-way ANOVAs performed to test for karyotypic effects on size-related traits and TL/WL scored in flies reared in three different cactus media

Trait	Source	df	Males		Females	
			MS	F	MS	F
TL	Karyotype	9	0.0156	9.35***	0.0140	7.77***
	Cactus	2	0.2255	135.27***	0.3290	182.14***
	Replicate	120	0.0016	1.85***	0.0018	1.74***
	Karyotype × Cactus	18	0.0018	1.07	0.0027	1.52
	Within	600	0.0009		0.0010	
WL	Karyotype	9	0.0091	11.11***	0.0074	6.41***
	Cactus	2	0.1600	196.07***	0.2048	177.50***
	Replicate	120	0.0008	1.46**	0.0012	1.44**
	Karyotype × Cactus	18	0.0010	1.24	0.0017	1.47
	Within	600	0.0006		0.0008	
WW	Karyotype	9	0.0093	9.98***	0.0110	8.47***
	Cactus	2	0.0486	52.46***	0.0984	76.10***
	Replicate	120	0.0009	1.42**	0.0013	1.47**
	Karyotype × Cactus	18	0.0012	1.26	0.0025	1.97*
	Within	600	0.0007		0.0009	
HW	Karyotype	9	0.0085	7.30***	0.0105	8.64***
	Cactus	2	0.3329	285.71***	0.3325	273.26***
	Replicate	120	0.0012	1.74***	0.0012	1.49**
	Karyotype × Cactus	18	0.0013	1.16	0.0018	1.45
	Within	600	0.0007		0.0008	
FW	Karyotype	9	0.0456	24.15***	0.0410	18.82***
	Cactus	2	0.2712	143.69***	0.3914	179.69***
	Replicate	120	0.0019	1.45**	0.0022	1.56***
	Karyotype × Cactus	18	0.0036	1.92*	0.0041	1.88*
	Within	600	0.0013		0.0014	
TL/WL	Karyotype	9	0.0050	4.57***	0.0030	3.95***
	Cactus	2	0.0290	28.31***	0.0280	38.40***
	Replicate	120	0.0010	1.57***	0.0007	1.25
	Karyotype × Cactus	18	0.0007	0.75	0.0009	1.37
	Within	600	0.0006		0.0006	

\*\*\* $P < 0.001$ , \*\*  $P < 0.01$ , \* $P < 0.05$ .

## Results

In the Appendix, we show the means and standard deviations of size and shape traits for males and females in each cactus host. In *O. ficus-indica*, *D. buzzatii* express the biggest size and increase wing loading, whereas in *O. vulgaris* and *T. terscheckii* there is not a clear trend. On one hand, in the former *D. buzzatii* express intermediate values for TL, HW and TL/WL ratio while in *T. terscheckii* intermediate values are observed for WW, WL and FW. In general males and females show comparable trends.

Both cactus species and karyotype affect significantly all size-related traits and wing loading, indicating that overall body size and shape are affected, not only by the cactus where the flies developed but also by the chromosomal inversions. Significant karyotype-by-cactus interactions are found only for WW in females and FW in both sexes (Table 1).

There is considerable variation among karyotypes in each cactus host in both sexes, which is most pronounced for wing loading (TL/WL index) (see the Appendix). Reaction norms of the different second chromosome karyotypes across the cactus host is plotted for wing loading (Figure 1). Individuals with homokaryotype *st/st* and those with heterokaryotype *st/jz<sup>3</sup>* and *st/jq<sup>7</sup>*

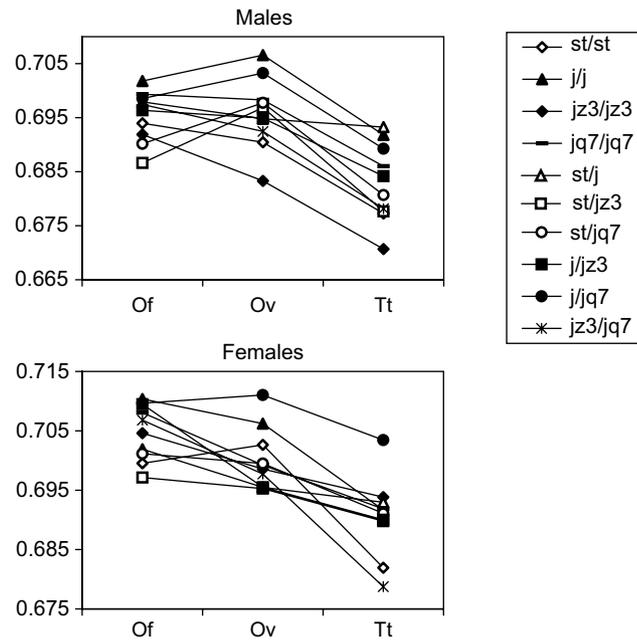
exhibit lowest wing loading in both sexes in *O. ficus-indica*, while individuals *jq<sup>7</sup>/jq<sup>7</sup>* shows lowest scores in males reared in *O. vulgaris* and *T. terscheckii*. On the other hand, *j/j* and *j/jq<sup>7</sup>* karyotypes exhibit highest values of wing loading in both sexes in *O. ficus-indica*.

Karyotypic correlations and the corresponding confidence intervals for each trait between pairs of cactus hosts in males and females are shown in Table 2. In general, positive and significant correlations across hosts ( $r_s$  differ from zero) are observed for most size-related and shape traits, except for six out of 18 values in males and for nine out of 18 values in females, though, confidence intervals are, in general, large and very close to one. However, it should be noted that the correlations across hosts for the TL/WL ratio in females are very low and different from one.

Karyotypic correlations (using the average in the three cactus media) among size-related traits are positive and most of them significant in both males and females (Table 3). Correlations among size-related traits and shape (TL/WL ratio) are positive but only significant for TL in both sexes and FW only in males.

Regression analysis of each trait on inversion dosage (Table 4) show that *2jq<sup>7</sup>* affects positively and consistently all size-related traits in both sexes and in all cactus

media, while it does not seem to affect the TL/WL ratio. Arrangement 2j also shows a size increasing effect for some traits (mainly TL) only in males and a positive



**Figure 1** Mean values for each second chromosome karyotypes for TL/WL index in each cactus host (Of: *Opuntia ficus-indica*; Ov: *Opuntia vulgaris*; Tt: *Trichocereus terscheckii*).

**Table 2** Karyotypic correlations between cactus host

	Of–Ov	Of–Tt	Ov–Tt
<i>Males</i>			
TL	<b>0.79 (0.95, 0.30)</b>	0.56 (0.88, –0.14)	<b>0.81 (0.96, 0.35)</b>
WL	<b>0.85 (0.97, 0.47)</b>	0.60 (0.90, –0.07)	0.58 (0.89, –0.10)
WW	<b>0.90 (0.98, 0.61)</b>	<b>0.71 (0.93, 0.12)</b>	<b>0.77 (0.95, 0.25)</b>
HW	<b>0.82 (0.96, 0.37)</b>	0.58 (0.89, –0.10)	0.43 (0.84, –0.29)
FW	<b>0.79 (0.95, 0.31)</b>	<b>0.75 (0.94, 0.21)</b>	<b>0.88 (0.97, 0.54)</b>
TL/WL	0.55 (0.88, –0.14)	<b>0.76 (0.94, 0.29)</b>	<b>0.72 (0.93, 0.15)</b>
<i>Females</i>			
TL	0.64 (0.91, –0.01)	0.52 (0.87, –0.19)	0.41 (0.83, –0.31)
WL	<b>0.66 (0.92, 0.04)</b>	<b>0.67 (0.92, 0.05)</b>	<b>0.65 (0.91, 0.004)</b>
WW	0.52 (0.87, –0.19)	<b>0.75 (0.94, 0.21)</b>	0.60 (0.90, –0.07)
HW	<b>0.74 (0.98, 0.19)</b>	0.58 (0.89, –0.10)	<b>0.75 (0.94, 0.21)</b>
FW	<b>0.90 (0.98, 0.60)</b>	<b>0.78 (0.95, 0.28)</b>	<b>0.87 (0.97, 0.52)</b>
TL/WL	0.30 (0.79, –0.43)	0.32 (0.80, –0.45)	0.30 (0.79, –0.43)

Confidence interval are given in parentheses. Of, *Opuntia ficus-indica*; Ov, *Opuntia vulgaris*; Tt, *Trichocereus terscheckii*. Bold-face values are different from zero ( $P < 0.05$ ).

**Table 3** Karyotypic correlations among traits: females are above the diagonal and males are below it

	TL	WL	WW	HW	FW	TL/WL
TL		0.68*	0.82**	0.93***	0.93***	0.68*
WL	0.74*		0.77**	0.84**	0.82**	0.09
WW	0.89***	0.74*		0.87**	0.88***	0.52
HW	0.90***	0.75*	0.94***		0.96***	0.47
FW	0.84**	0.52	0.89***	0.89***		0.50
TL/WL	0.68*	0.07	0.51	0.52	0.66*	

\*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ .

effect on TL/WL index in *T. terscheckii* (both sexes) and in *O. ficus-indica* (males). Inversion 2jz<sup>3</sup> shows negative effects on some traits but not in all cactus species and a negative effect on TL/WL index in *O. vulgaris* in both sexes. Arrangement 2st exhibits a negative effect on both WL in males reared in *T. terscheckii* and HW in females reared in *O. vulgaris* and a negative effect on TL/WL index in females reared in *O. ficus-indica* (Table 4). The proportion of additive variance explained by chromosome inversions (ie,  $R^2$  for our regression model, see above) is generally higher in both *Opuntias* cacti (Of and Ov) than in cardón (Tt) (Table 4).

## Discussion

The main findings of the present study are that polymorphic second chromosome inversions affect body size and shape (wing loading) in *D. buzzatii* and that variation in wing loading across natural breeding substrates has a genetic basis that can be partially explained by the inversion polymorphism.

The observed general effects on size and shape (wing loading) depend on the media where flies were reared. *D. buzzatii* flies emerging on *Opuntia ficus-indica* were larger than those reared in *T. terscheckii* and *O. vulgaris*. This may be related to a more restricted supply of nutrients in *O. vulgaris* (Fernández Iriarte et al, 1999) and *T. terscheckii*. Thus, the nutritional quality of the different breeding sites may drastically affect size and shape traits, as shown by Starmer and Wolf (1989) for wing loading in *D. mulleri*, which decreased in response to increased

**Table 4** Partial regression coefficients ( $\beta'$ ) and statistics ( $R^2$  and  $F$ ) of overall multiple regression are shown for regressions of size-related traits and TL/WL on the dosage of each second chromosome arrangement in each cactus medium

Trait	Cactus	$\beta'$				$R^2$	$F$
		$st$	$j$	$jz^3$	$jq^7$		
TL(M)	Of				0.34***	0.13	12.46***
	Ov		0.24***		0.35***	0.12	17.82***
	Tt		0.33***		0.23***	0.11	15.61***
TL(F)	Of		0.13*		0.38***	0.17	16.91***
	Ov			-0.27***	0.22***	0.15	23.78***
	Tt				0.21***	0.04	5.51**
WL(M)	Of				0.40***	0.14	20.74***
	Ov				0.27***	0.07	19.55***
	Tt	-0.16*				0.03	3.28*
WL(F)	Of				0.31***	0.10	27.05***
	Ov			-0.23***	0.26***	0.16	23.90***
	Tt				0.19**	0.04	9.15**
WW(M)	Of				0.33***	0.11	29.82***
	Ov				0.32***	0.13	19.58***
	Tt		0.17***		0.26***	0.07	8.86***
WW(F)	Of				0.37***	0.12	17.38***
	Ov			-0.25***	0.32***	0.21	33.54***
	Tt				0.20**	0.04	4.73**
HW(M)	Of		0.14*		0.42***	0.16	23.17***
	Ov		0.22***		0.33***	0.10	33.54***
	Tt			-0.18***		0.03	4.73***
HW(F)	Of				0.51***	0.23	37.44***
	Ov	-0.16*		-0.33***	0.15***	0.16	14.98***
	Tt				0.17**	0.03	3.89*
FW(M)	Of			-0.28***	0.39***	0.32	38.27***
	Ov				0.61***	0.34	65.14***
	Tt		0.28**		0.39***	0.16	23.48***
FW(H)	Of			-0.24***	0.45***	0.33	60.68***
	Ov				0.62***	0.30	35.90***
	Tt			-0.26***		0.11	9.97***
TL/WL(M)	Of		0.19**			0.04	4.79**
	Ov			-0.22**		0.07	7.68***
	Tt		0.25***			0.10	9.95***
TL/WL(F)	Of	-0.25***				0.05	7.35***
	Ov			-0.15*		0.02	3.08*
	Tt		0.18**			0.02	3.65*

M, males; F, females. Of, *Opuntia ficus-indica*; Ov, *Opuntia vulgaris*; Tt, *Trichocereus terscheckii*. \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ .

larval density. In a previous paper we have shown that when *D. buzzatii* is reared on *O. ficus-indica*, flies have a shorter developmental time than on *O. vulgaris* and on *T. terscheckii* (Fernández Iriarte and Hasson, 2000). In this sense, an inferior nutritional quality may be the cause of the extended developmental time and the small wing loading of flies emerging from vials with seminatural media prepared with Ov and Tt.

Genetic variability in plasticity among cactus species is not a significant component of variation for size-related traits, but we have shown that this component was significant for life-history traits such as developmental time and viability (Fernández Iriarte and Hasson, 2000). Although FW showed a significant karyotype  $\times$  cactus interaction, the correlations estimated for this trait among environments (cactus hosts) were not different from one. These results suggests that the same set of genes affect variation in size-related traits in different cactus hosts. However, in females the wing loading showed a slightly different picture since karyotypic correlations among environments were different from one. These observations suggest that in females different sets of loci may affect variation in wing loading across cactus hosts.

Wing loading is related to flight cost and is probably an important target of natural selection in *Drosophila* (Stalker, 1980; Starmer and Wolf, 1989; Barker and Krebs, 1995; Azevedo et al, 1998; Loeschcke et al, 1999). Moreover, it has been shown that variation in this compound trait has a significant genetic basis (Azevedo et al, 1998). Likewise, according with past directional selection on wing loading, the heritability of this trait in *Drosophila buzzatii* is lower than at least one of its components (TL) (Loeschcke et al, 1999). Evidence of a  $G \times E$  interaction for wing loading was detected when flies developed at different temperatures in *D. buzzatii* (Thomas and Barker, 1993; Loeschcke et al, 1999) and *D. melanogaster* (David et al, 1994). Similarly, temperatures plus nutritional constraints were also shown to affect wing loading in *D. aldrichi* and *D. buzzatii* (Barker and Krebs, 1995). In the latter study, the reaction norm of wing loading was due to the compound effect of temperature and nutritional quality (Barker and Krebs, 1995). Thus, available evidence suggest that reduced wing loading may be a response to limitations of food supply that may cause strong competition among larvae (Starmer and Wolf, 1989). Therefore, differences in nutritional quality may be a plausible explanation for the wing loading

variation among cactus hosts detected in the present study.

The effect of inversions on shape (measured as wing loading) is a new finding for the chromosomal polymorphism of *D. buzzatii*. Reaction norms of karyotypes differ in the direction of response across cacti for wing loading (Figure 1 and Tables 2 and 4). For instance, arrangements 2st and 2jz<sup>3</sup> showed a decreasing effect on wing loading in both *Opuntias*, whereas 2j affected positively this trait mainly in *T. terschekii*.

In *D. buzzatii* natural populations frequencies variation of arrangements 2st and 2j are, respectively, positively and negatively correlated with temperature in Australia (Knibb and Barker, 1988) and 2st is negatively correlated with latitude and with altitude in South American populations (Hasson *et al*, 1995; Rodríguez *et al*, 2000). In addition, there is evidence that wing loading decreases linearly with latitude and that reduced wing loading has an adaptive advantage for flying at cold temperatures (Stalker, 1980; David *et al*, 1994). Thus we may ask whether clinal variation of inversion frequencies along latitudinal and thermal gradients can be accounted for by the effect of inversions on wing loading. However 2st, an arrangement that decreases wing loading, has a negative correlation with latitude, exactly the opposite to that expected.

Wing loading can be also related to dispersal costs and dispersal distances (Starmer and Wolf, 1989). Thus, lower values appear to occur among forms that must fly relatively longer distances to find breeding and feeding locations and higher wing loading might be selectively advantageous if shorter flight (hovering) is important at warm temperatures (Starmer and Wolf (1989) and citations therein). Thus, the small wing loading detected for 2st is expected to confer a better flight performance in natural populations where resources are rare, or more scattered, such as the rotting stems of *T. terschekii* in the arid zones of South America. The high viability (Fernández Iriarte and Hasson, 2000) and the low wing loading detected for 2st, the ancestral gene order in *D. buzzatii* (Ruiz and Wasserman, 1993), in a cactus host that belongs to the group of putative ancestral host plants of the *D. buzzatii* cluster (Hasson *et al*, 1992b), are, perhaps, aspects related to the evolutionary history of host plant utilization in this species. Thus, the ancestral gene order characterized by its small wing loading may be better adapted to exploit a host plant characterized by a patchy and scattered distribution. On the other hand, derived arrangements such as 2j with their relatively large wing loading, may be advantageous in exploiting more predictable resources such as *Opuntias*. Moreover, this characteristic may be a plausible explanation for the success of an inversion that has increased in frequency in relatively recent times (Cáceres *et al*, 2001) probably following the expansion of prickly pears.

Genetic variation in the ratio TL/WL is primarily due to TL (significant positive karyotypic correlation, Table 3). Thus, selection to decrease wing loading will decrease TL, but with not correlated selection for WL as reported by Thomas and Barker (1993). However, Loeschcke *et al* (1999) observed another picture since variation in wing loading was apparently due to variation in distal wing length (not considered in our studies) but they found also that wing loading has a marginally significant correlation with thorax length. Since these traits could

vary along a latitudinal cline (Barker and Krebs, 1995; Azevedo *et al*, 1998) the patterns of genetic correlations between size and shape traits could be different among populations.

Regression analysis show that both 2jq<sup>7</sup> and 2j increase the size of adult flies in general agreement with previous studies (Ruiz *et al*, 1991; Hasson *et al*, 1992a; Norry *et al*, 1995b; Fernández Iriarte and Hasson, 2000), but only the former had a consistent effect in all cactus hosts (Table 4). Conversely, inversions 2st and 2jz<sup>3</sup> decreased size. However, it is important to note that in other studies it has been shown that the latter has a positive effect on body size (Ruiz *et al*, 1991; Hasson *et al*, 1992a) which along with the present results suggest that the effects of 2jz<sup>3</sup> on size-related traits may depend on the rearing medium, that is, a genotype by environment interaction not detected previously. Perhaps, the most interesting observation is the strong effect observed for 2jq<sup>7</sup> on all traits studied. This inversion is either rare or absent in natural populations of the New World (Hasson *et al*, 1995) but increased substantially in frequency during the colonization of the Old World (Fontdevila, 1989). The finding that 2jq<sup>7</sup> strongly increases body size in different breeding resources is particularly interesting because this inversion is known to extend developmental time in all natural breeding resources tested (Fernández Iriarte and Hasson, 2000).

Karyotypic correlations of TL/WL index across host plants are slightly smaller in females than in males suggesting that males have a weaker response than females to environmental variation. Similar findings have been reported in *D. melanogaster* for several morphological traits (Cowley and Atchley, 1990) and for body weight among flies raised with different larval foods (Hillesheim and Stearns, 1991). A similarly asymmetrical response of the sexes, measured as wing asymmetry, has been reported in *D. buzzatii* populations exposed to temperature stress, suggesting that females are less tolerant to stress than males (Loeschcke *et al*, 1994).

We have presented here new evidence concerning the effects of the adaptive inversion polymorphism in *D. buzzatii*; however, the characterization of QTLs associated to size and shape traits, mapping on the second chromosome of *D. buzzatii*, would allow a better understanding of the complex interaction among the exophenotype, the genotype and fitness associated with the inversion polymorphism in *Drosophila*.

## Acknowledgements

We thank M Santos for helpful discussions and constructive comments on an earlier draft of this manuscript. We thank two anonymous reviewers for valuable suggestions. This work was supported by Universidad de Buenos Aires, CONICET and Universidad Nacional de Mar del Plata grants awarded to EH. PFI is fellow and FN and EH are members of Carrera del Investigador Científico of CONICET (Argentina).

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## Appendix

The mean of size-related traits and shape of *Drosophila buzzatii* second chromosome karyotypes in each cactus medium is summarized in Table 5.

**Table 5** Mean of size-related traits and shape (in mm) of *Drosophila buzzatti* second chromosome karyotypes in each cactus medium

Trait	Cactus	st/st	jj/j	jz <sup>3</sup> /jz <sup>3</sup>	jq <sup>7</sup> /jq <sup>7</sup>	st/j	st/jz <sup>3</sup>	st/jq <sup>7</sup>	jj/z <sup>3</sup>	jj/jq <sup>7</sup>	jz <sup>3</sup> /jq <sup>7</sup>	Total
<i>(a) Males</i>												
TL	Of	0.991 (0.033)	1.000 (0.024)	0.991 (0.026)	1.019 (0.028)	0.988 (0.024)	0.981 (0.033)	1.013 (0.024)	1.011 (0.029)	1.015 (0.035)	1.013 (0.027)	1.002 (0.031)
	Ov	0.941 (0.038)	0.959 (0.027)	0.939 (0.022)	0.965 (0.031)	0.941 (0.032)	0.944 (0.027)	0.971 (0.035)	0.956 (0.031)	0.992 (0.029)	0.949 (0.038)	0.956 (0.034)
	Tt	0.932 (0.014)	0.959 (0.015)	0.919 (0.012)	0.947 (0.014)	0.947 (0.015)	0.939 (0.013)	0.955 (0.012)	0.965 (0.015)	0.973 (0.013)	0.947 (0.015)	0.948 (0.036)
WL	Of	1.428 (0.029)	1.425 (0.030)	1.432 (0.026)	1.457 (0.025)	1.416 (0.026)	1.429 (0.034)	1.468 (0.028)	1.451 (0.033)	1.454 (0.035)	1.452 (0.026)	1.441 (0.033)
	Ov	1.363 (0.031)	1.357 (0.032)	1.374 (0.030)	1.382 (0.043)	1.355 (0.029)	1.354 (0.032)	1.391 (0.029)	1.377 (0.040)	1.410 (0.036)	1.371 (0.039)	1.373 (0.038)
	Tt	1.376 (0.032)	1.386 (0.031)	1.370 (0.040)	1.381 (0.041)	1.366 (0.030)	1.386 (0.038)	1.404 (0.047)	1.410 (0.032)	1.412 (0.047)	1.397 (0.040)	1.389 (0.041)
WW	Of	0.970 (0.026)	0.969 (0.025)	0.973 (0.026)	0.990 (0.015)	0.972 (0.015)	0.961 (0.022)	0.988 (0.019)	0.978 (0.027)	0.985 (0.025)	0.982 (0.022)	0.977 (0.024)
	Ov	0.946 (0.023)	0.940 (0.025)	0.946 (0.023)	0.970 (0.029)	0.947 (0.027)	0.942 (0.026)	0.970 (0.023)	0.948 (0.033)	0.982 (0.023)	0.947 (0.031)	0.954 (0.029)
	Tt	0.947 (0.028)	0.953 (0.031)	0.935 (0.030)	0.954 (0.027)	0.948 (0.022)	0.943 (0.026)	0.966 (0.021)	0.959 (0.029)	0.971 (0.023)	0.960 (0.027)	0.953 (0.028)
HW	Of	0.932 (0.018)	0.933 (0.017)	0.925 (0.019)	0.945 (0.016)	0.927 (0.019)	0.913 (0.023)	0.946 (0.015)	0.931 (0.019)	0.946 (0.023)	0.944 (0.021)	0.934 (0.021)
	Ov	0.866 (0.036)	0.883 (0.021)	0.870 (0.023)	0.887 (0.028)	0.863 (0.027)	0.867 (0.025)	0.885 (0.022)	0.876 (0.029)	0.903 (0.029)	0.876 (0.027)	0.877 (0.029)
	Tt	0.871 (0.019)	0.876 (0.032)	0.862 (0.029)	0.869 (0.025)	0.876 (0.022)	0.871 (0.022)	0.888 (0.024)	0.883 (0.024)	0.894 (0.032)	0.880 (0.027)	0.877 (0.027)
FW	Of	0.402 (0.016)	0.405 (0.015)	0.390 (0.018)	0.423 (0.012)	0.402 (0.012)	0.388 (0.016)	0.416 (0.014)	0.399 (0.014)	0.411 (0.016)	0.413 (0.013)	0.405 (0.018)
	Ov	0.369 (0.014)	0.386 (0.015)	0.370 (0.012)	0.399 (0.014)	0.376 (0.015)	0.373 (0.013)	0.386 (0.012)	0.376 (0.015)	0.399 (0.013)	0.384 (0.015)	0.382 (0.017)
	Tt	0.371 (0.017)	0.387 (0.016)	0.371 (0.014)	0.386 (0.015)	0.385 (0.012)	0.374 (0.011)	0.392 (0.016)	0.378 (0.016)	0.394 (0.017)	0.390 (0.015)	0.383 (0.017)
TL/WL	Of	0.694 (0.021)	0.702 (0.017)	0.692 (0.018)	0.699 (0.021)	0.698 (0.017)	0.687 (0.018)	0.690 (0.019)	0.696 (0.017)	0.699 (0.024)	0.697 (0.014)	0.695 (0.019)
	Ov	0.690 (0.019)	0.707 (0.015)	0.683 (0.016)	0.698 (0.017)	0.695 (0.021)	0.697 (0.019)	0.698 (0.019)	0.695 (0.017)	0.703 (0.017)	0.692 (0.016)	0.696 (0.018)
	Tt	0.677 (0.016)	0.692 (0.024)	0.671 (0.015)	0.686 (0.014)	0.693 (0.017)	0.678 (0.020)	0.681 (0.019)	0.684 (0.014)	0.689 (0.024)	0.678 (0.018)	0.683 (0.019)
<i>(b) Females</i>												
TL	Of	1.075 (0.027)	1.092 (0.029)	1.080 (0.030)	1.101 (0.027)	1.066 (0.020)	1.069 (0.030)	1.106 (0.024)	1.089 (0.029)	1.113 (0.016)	1.098 (0.028)	1.089 (0.030)
	Ov	1.031 (0.038)	1.026 (0.038)	1.007 (0.039)	1.052 (0.036)	1.025 (0.034)	1.005 (0.033)	1.032 (0.036)	1.002 (0.036)	1.064 (0.037)	1.015 (0.035)	1.026 (0.040)
	Tt	0.998 (0.052)	1.014 (0.031)	1.014 (0.036)	1.035 (0.045)	1.018 (0.032)	1.022 (0.031)	1.027 (0.042)	1.021 (0.029)	1.048 (0.050)	1.009 (0.050)	1.021 (0.042)
WL	Of	1.538 (0.033)	1.537 (0.033)	1.533 (0.026)	1.556 (0.041)	1.519 (0.034)	1.534 (0.037)	1.578 (0.035)	1.536 (0.047)	1.569 (0.041)	1.553 (0.040)	1.545 (0.040)
	Ov	1.467 (0.034)	1.453 (0.048)	1.441 (0.036)	1.504 (0.049)	1.474 (0.047)	1.445 (0.042)	1.475 (0.048)	1.441 (0.039)	1.496 (0.043)	1.455 (0.045)	1.465 (0.048)
	Tt	1.462 (0.045)	1.467 (0.047)	1.462 (0.037)	1.496 (0.050)	1.469 (0.035)	1.482 (0.032)	1.487 (0.067)	1.481 (0.047)	1.490 (0.060)	1.487 (0.057)	1.478 (0.050)
WW	Of	1.039 (0.023)	1.041 (0.031)	1.050 (0.017)	1.056 (0.020)	1.024 (0.026)	1.026 (0.025)	1.060 (0.016)	1.033 (0.032)	1.060 (0.029)	1.052 (0.023)	1.044 (0.028)
	Ov	1.006 (0.026)	1.000 (0.040)	0.987 (0.028)	1.043 (0.036)	1.008 (0.031)	0.992 (0.037)	1.015 (0.036)	0.987 (0.031)	1.041 (0.030)	0.995 (0.029)	1.007 (0.038)
	Tt	0.997 (0.046)	1.012 (0.037)	1.006 (0.025)	1.023 (0.033)	1.005 (0.030)	1.010 (0.024)	1.019 (0.037)	1.005 (0.026)	1.022 (0.039)	1.010 (0.044)	1.011 (0.035)
HW	Of	0.974 (0.014)	0.977 (0.019)	0.967 (0.018)	0.990 (0.019)	0.964 (0.018)	0.957 (0.015)	0.998 (0.022)	0.969 (0.028)	0.995 (0.015)	0.995 (0.023)	0.979 (0.024)
	Ov	0.918 (0.029)	0.926 (0.028)	0.898 (0.029)	0.942 (0.035)	0.914 (0.027)	0.907 (0.030)	0.924 (0.034)	0.913 (0.027)	0.949 (0.024)	0.916 (0.032)	0.921 (0.033)
	Tt	0.910 (0.031)	0.918 (0.027)	0.908 (0.027)	0.925 (0.032)	0.914 (0.023)	0.916 (0.028)	0.918 (0.045)	0.918 (0.021)	0.932 (0.036)	0.916 (0.035)	0.918 (0.031)
FW	Of	0.439 (0.014)	0.441 (0.017)	0.421 (0.013)	0.456 (0.015)	0.425 (0.013)	0.430 (0.016)	0.451 (0.016)	0.428 (0.017)	0.452 (0.014)	0.445 (0.011)	0.439 (0.019)
	Ov	0.402 (0.019)	0.414 (0.018)	0.393 (0.016)	0.430 (0.018)	0.402 (0.020)	0.394 (0.013)	0.409 (0.017)	0.399 (0.015)	0.426 (0.019)	0.409 (0.016)	0.408 (0.021)
	Tt	0.406 (0.020)	0.415 (0.015)	0.402 (0.013)	0.422 (0.018)	0.414 (0.012)	0.408 (0.012)	0.419 (0.019)	0.410 (0.012)	0.419 (0.023)	0.410 (0.017)	0.413 (0.017)
TL/WL	Of	0.700 (0.020)	0.710 (0.016)	0.705 (0.017)	0.708 (0.018)	0.702 (0.017)	0.697 (0.017)	0.701 (0.016)	0.709 (0.018)	0.710 (0.017)	0.707 (0.016)	0.705 (0.017)
	Ov	0.703 (0.019)	0.706 (0.017)	0.699 (0.018)	0.699 (0.011)	0.695 (0.018)	0.695 (0.015)	0.700 (0.016)	0.695 (0.020)	0.711 (0.022)	0.698 (0.013)	0.700 (0.018)
	Tt	0.682 (0.022)	0.692 (0.016)	0.694 (0.022)	0.692 (0.014)	0.692 (0.014)	0.690 (0.019)	0.691 (0.013)	0.690 (0.018)	0.703 (0.015)	0.679 (0.014)	0.691 (0.018)

Standard deviations are given in parentheses. TL, thorax length; WL, wing length; WW, wing width; HW, head width; FW, face width; TL/WL, thorax/wing length ratio; Of, *Opuntia ficus-indica*; Ov, *Opuntia vulgaris*; Tt, *Trichocereus terscheckii*.