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ORIGINAL PAPER

Desiccation resistance along an aridity gradient in the cactophilic fly *Drosophila buzzatii*: sex-specific responses to stress

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Abstract Stress resistance characters are valuable tools for the study of acclimation potential, adaptive strategies and biogeographic patterns in species exposed to environmental variability. Water stress is a challenge to terrestrial arthropods because of their small size and relatively high area: volume ratio. Fruit flies have been investigated to record adaptive morphological and physiological traits, as well as to test their responses to stressful factors. In this study, we investigate the ability to cope with water stress, by examining variation in desiccation resistance in a species that lives mainly in desert lands. Specifically, we explored the genetic and ecological basis of desiccation resistance in populations of *Drosophila buzzatii* from Northern Argentina. We used a common garden experiment with desiccation treatments on a number of isofemale lines from four populations along an aridity gradient. Our results revealed significant among-population differentiation and substantial amounts of genetic variation for desiccation resistance. We also detected significant genotype-by-environment and genotype-by-sex interactions indicative that desiccation resistance responses of the lines assayed were environment- and sex-specific. In addition, we observed clinal variation in female desiccation resistance along gradients of altitude, temperature and humidity; that desiccation resistance is a sexually dimorphic trait, and that sexual dimorphism increased along the aridity and altitudinal gradients. Based on current evidence, we propose that the observed sex-specific responses

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may reflect different life history traits, and survival and reproductive strategies in different ecological scenarios.

Keywords Intraspecific variation · Desiccation resistance · Clines · Sexual dimorphism · *Drosophila*

Introduction

The study of stress resistance characters is a useful approach to understand adaptive strategies, as well as biogeographic patterns, in species exposed to spatial and temporal variation in environmental variables. The beneficial acclimation hypothesis proposes that the extent to which organisms can respond to diverse environmental conditions directly affects their fitness (Leroi et al. 1994). In this sense, phenotypic changes induced by the environment can result from phenotypic plasticity or local adaptation driven by genetic differentiation. Phenotypic plasticity has been recognized as a major force promoting diversification of traits with evolutionary relevance (West-Eberhard 1989; Schlichting and Pigliucci 1998; Fordyce 2006). Furthermore, evidence for local adaptation has been suggested as a frequent outcome of long-term divergent environmental pressures, and particularly, has been proposed as an explanation of the responses to stressful conditions in *Drosophila* (Hoffmann et al. 2001, 2002).

To investigate how stress could trigger the evolution of specialized ecotypes, Bradshaw and Hardwick (1989) propose an approach based on two levels of analysis: ecological and evolutionary. The authors claim that if stress occurs frequently and alternating with non-stressful conditions, an optimal strategy could be the generalist one, based on phenotypic plasticity. On the other hand, if none of the different genotypes shows the maximum yield in all environments, selection would favor alternative genotypes in different patches (Thomas and Barker 1993; Ungerer et al. 2003). Therefore, reaction norms depend on the periodicity of environmental change, the spatial or temporal nature of environmental variation, and the intensity of selection. Genetic variation in reaction norms has important evolutionary implications by adding to the maintenance of organisms' ability for acclimation, as genotype-by-environment interactions evince (Carreira et al. 2006; Goenaga et al. 2010).

Deserts impose particularly challenging conditions that translate into strong selective pressures on water and nutritional balance. Insects and other terrestrial arthropods are especially susceptible to water loss because of their small size and relatively high area: volume ratio (Gibbs et al. 1997). Investigations in fruit flies searching for adaptive morphological and physiological traits and testing for acclimation ability to desert conditions have been very prolific. Comparative studies in the genus *Drosophila* revealed that traits playing a role in water economy like reduced permeability, lower water excretion rate, high temperatures and dehydration tolerance differ between species living in arid environments and counterparts facing mesic climate regimes (Hadley 1994; Gibbs and Matzkin 2001; Gibbs et al. 2003; Marron et al. 2003; Matzkin et al. 2007).

Typical studies of desiccation resistance have mainly consisted of interspecific or interpopulation comparisons in *D. melanogaster*, the most cosmopolitan and well-known species of the genus, and allied species (Hoffmann and Harshman 1999; Gibbs et al. 2003). However, to fully understand responses to desiccation stress, it would be more rewarding to investigate intra-specific and interspecific variation in related traits in species native to desert lands (Loeschcke et al. 2004).

Interpopulation differentiation can occur at different geographic scales in response to ecological factors. In this sense, geographic gradients provide the opportunity to assess stress responses through comparisons among populations inhabiting contrasting abiotic conditions, e.g. along an aridity gradient. The chief aim of the present study is to investigate the genetic and ecological basis of variation in desiccation resistance along an aridity gradient in a desert inhabitant, the cactophilic *Drosophila buzzatii*. This species occurs throughout Northern and Western Argentina and its distribution is strongly associated to the presence of prickly pear cacti of the genus *Opuntia* that provide the main breeding and feeding resources (Hasson et al. 1992). *Opuntia* cacti mainly occur in arid and semiarid regions. However, the introduction of the cultivated *O. ficus-indica* into mesic and humid regions may account for the widespread distribution of *D. buzzatii*, which from the arid zones of Western and North Western Argentina has extended to Central and North Eastern Argentina (Fontdevila 1989), suggesting a strong colonizing potential that allowed its access to a wide spectrum of climatic regions.

The rationale of our study is that populations living under similar thermal conditions (mean annual temperature) but varying in the degree of aridity may represent a natural laboratory in which controlling the former may allow uncovering the effect of water shortage on flies. In view of the broad distribution of *D. buzzatii*, we also expect to find phenotypic plasticity and/or genetic variation in desiccation resistance. In addition, we measured thorax length as a proxy of body size since it plays an important role in desiccation resistance in other desert dwellers of the genus, e.g. *D. nigrospiracula* (Matzkin et al. 2007). Body size is highly correlated with some of the putative mechanisms underlying desiccation resistance, such as carbohydrate storage and low water loss rate. Our main hypothesis is that the effect of desiccation depends on the geographic origin of the flies, i.e. flies from more arid areas are expected to be more resistant than those from humid areas.

Materials and methods

Fly collections and maintenance of stocks

We sampled four populations of *D. buzzatii* along an aridity gradient that decreases towards the East, at four sites in Northern Argentina located at roughly the same latitude. Location, climate parameters and an aridity index calculated according to Tieleman et al. (2003) for each collecting site are shown in Table 1.

At each site, we collected adult flies, using the presence of *Opuntia* sp. as a signal of *D. buzzatii*'s presence. Flies were captured by net sweeping on fermented banana baits, taken to the laboratory and sorted by sex. Inseminated females collected in each locality were placed in individual vials to establish sets of isofemale lines by rearing their progenies in commercial instant smashed potato hydrated with a water solution of Nipagin (p-hydroxybenzoic acid methyl ester) as antifungal agent and supplemented with baker yeast. We identified lines to species by inspection of the genitalia of one male progeny and the polytene chromosomes of third instar larvae. These procedures are necessary since females cannot be distinguished from species such as *D. koepferae* or *D. antonietae* that coexist with *D. buzzatii* in some of the localities sampled for the present study (Hasson et al. 1992; Manfrin and Sene 2006). All lines were maintained for five generations in glass vials containing instant smashed potato medium at low density and under standard conditions of light and temperature (12:12 light/dark at 25 °C).

Table 1 Geographical coordinates and climate estimates of four collection sites for populations of *D.buzzatii* sampled in the present study

Site	Montecarlo	Palo Santo	Ing. Juárez	Tilcara
Longitude (West)	54°46′	59°20′	61°51′	65°23′
Latitude (South)	26°34′	25°33′	23°54′	23°34′
Altitude (m asl)	175	249	508	2,461
Mean annual rainfall (mm)	2,302	1,498	740	141
Maximum temperature (°C)	32.9	33.8	35.4	29.8
Minimum temperature (°C)	11.3	11.4	9.9	4
Thermal amplitude[a] (°C)	21.6	22.4	25.5	25.8
Aridity index	1,949	1,455	640	161
Mean annual temperature (°C)	21	22	23	19
Saturation vapour pressure	2.48	2.64	2.81	2.20

[a] According to Strahler and Strahler (1989), in climatic series, annual thermal amplitude is the difference between mean minimum temperature of the coldest month and the mean maximum temperature of the hottest month

Experimental design

We randomly chose ten isofemale lines of each population from the original set. For each line, 200 sexually mature flies were released in egg-collection chambers (three chambers per isofemale line). We used medium size Petri dishes containing an egg laying medium (3 g agar and 175 ml distilled water) for egg collection that were replaced after 24 h. Dishes were incubated until egg hatching (approximately 36 h) at 25 °C. Eggs were allowed to hatch and then we transferred batches of 30 first-instar larvae to culture vials containing laboratory medium. Larvae were raised at 25 ± 1 °C with 12:12 light/dark until emergence of adult flies. This procedure allowed us to obtain flies reared under controlled conditions of density, temperature, humidity and photoperiod to minimize environmental variation.

Groups of ten 5 ± 1 day-old males and females of an isofemale line were released in 30 ml vials, and exposed to three desiccation treatments: high desiccation (3 g Fresh Drierite desiccant), medium desiccation (0.5 g Fresh Drierite desiccant) and low desiccation (no desiccant). A polyethylene sponge kept the flies in the upper half of the vial, separated from the desiccant, and sealed with Parafilm. We checked flies for death at hourly intervals, as indicated by failure to right themselves or move their legs when vials were tapped or inverted. The response variable we measured was LT 50 (lethal tolerance time, in hours, at which 50 % of flies had died).

For body size measurements, we randomly chose ten females and ten males of each line and population to measure thorax length (Hasson et al. 1992). To this end we captured images of flies using a binocular microscope (10×) and an attached digital camera connected to a computer using public domain Scion software (available at http://www.scioncorp.com).

In addition, we selected lines showing the highest and lowest LT50 scores to make crosses between lines to examine the genetic basis of desiccation resistance. To this end, we assessed desiccation resistance in F1 flies along with their corresponding parental lines.

Statistical analysis

Our experimental design corresponds to a model of factorial analysis of variance (Sokal and Rohlf 1981), which allowed partition of the total variation into LT50 according to the model:

$$y = \mu + P + S + T + L(P) + P \times T + P \times S + S \times T + T \times L(P) + S \times L\,(P) + P \times S \times T + \varepsilon$$

where μ is the overall mean, P (population), T (treatment) and S (sex) are fixed effects, $L(P)$ is the random effect of line (nested in population) and ε is the error variance.

We performed additional ANOVAs for each population separately to further investigate the relative contribution of line and sex factors to desiccation resistance according to the model:

$$y = \mu + L + S + L \times S + \varepsilon$$

where a significant L effect may be interpreted as the genetic component of phenotypic variance, since lines (families) may be considered different genotypes (David et al. 2005). A significant $L \times S$ interaction, which denotes variation in sexual dimorphism across genotypes, may be construed as an estimate of the genotype-by-sex interaction ($G \times S$) and can be interpreted as a deviation from the perfect genetic correlation between sexes (Muir et al. 1992). Only flies exposed to Drierite desiccant treatments were included in these ANOVAs.

We also estimated the cross-sex genetic correlation in each population using the equation:

$$r_{mf} = \mathrm{Cov}_{mf}/\sigma_m\sigma_f,$$

where Cov_{mf} represents the covariance between males and females, calculated from bivariate ANOVAs, and σ_m and σ_f are the square roots of the among-line variance component obtained from univariate ANOVAs performed for males and females separately. An r_{mf} equal to one may be considered an indication that the same sets of genes are involved in trait variation in both sexes, while an r_{mf} close to zero suggests that the sets of genes affecting trait variation in males and females are largely independent (Falconer 1952). We used ANOVA results to infer whether a given correlation was significantly different from zero or one: cross-sex correlations are significantly different from 1 if the line-by-sex interaction term from the pooled analysis is significant, and are significantly different from 0 if the among-line variance from the same analysis is significant (Vieira et al. 2000).

To further analyze the possible effect of environmental variables on phenotypic inter-population variation, we performed Linear Regressions of mean LT50 in females, mean LT50 in males and the female LT50: male LT50 ratio (as a surrogate of sexual dimorphism), using lines as experimental units, on each of the climatic variables listed in Table 1.

The same ANOVA design described above was used to analyze thorax length data.

Finally, desiccation resistance data for parental lines (genotypes) and the corresponding F1 hybrid progeny in each inter-strain cross were analyzed by means of linear regressions. To this end, we assigned numbers 0 and 2 to each parental genotype and 1 to the corresponding hybrids and regressed LT50 (dependent variable) on genotype. This methodology allows partitioning total phenotypic variance into additive (given by the proportion of variance accounted for by the regression) and non-additive (dominance or epistasis, given by the proportion of variation not explained by the regression) components of phenotypic variance.

All statistical analyses were performed using the GLM procedure implemented in the STATISTICA 7.0 software package (StatSoft 2007).

Results

The overall ANOVA revealed significant differences between males and females, among populations, treatments and lines (within populations). Moreover, the line-by-sex and line-by-treatment interactions were significant and the population-by-sex interaction was marginally significant (Table 2). Particularly, flies from Juárez were significantly the least resistant in all treatments (Fig. 1). Though, on average, females were significantly more resistant than males, and the trend was consistent across all populations, differences between sexes were significant only in Tilcara and Juárez (Fig. 2). Control flies (not exposed to a desiccation treatment) (mean = 38.73 h, SE = 0.61) survived longer than flies exposed to both desiccation treatments: 0.5 g (mean = 22.69 h, SE = 0.39) and 3 g of Drierite (mean = 22.11 h, SE = 0.36), which showed no statistical differences.

To explore desiccation effects on each population we ran separate ANOVAs, considering line and sex effects on survival. A common feature of these analyses was the significant line-by-sex interaction, which suggests that the response to desiccation of individual lines was sex-specific (Table 3; Fig. 3a–d).

Linear regressions of desiccation resistance on environmental variables were not significant in males. In contrast, desiccation resistance was negatively and highly significantly correlated with several climatic variables like maximum mean temperature ($R^2 = 0.25$, $B = -0.49$, $p = 0.001$), mean annual temperature ($R^2 = 0.24$, $B = -0.49$, $p = 0.001$) and saturation vapour pressure (SVP) ($R^2 = 0.25$, $B = -0.5$, $p = 0.001$) in females. Also, female LT50 was significantly and positively correlated with altitude ($R^2 = 0.1$, $B = 0.31$, $p = 0.04$). Further regression analysis using the female LT50: male LT50 ratio as dependent variable revealed negative and highly significant correlations with rainfall ($R^2 = 0.1$, $B = -0.31$, $p = 0.004$), minimal temperature ($R^2 = 0.13$, $B = -0.37$, $p = 0.01$) and aridity index ($R^2 = 0.1$, $B = -0.33$, $p = 0.03$), and a significant and positive relationship with altitude ($R^2 = 0.13$, $B = 0.36$, $p = 0.02$).

In addition, we calculated the cross-sex genetic correlation (r_{mf}) to assess the extent to what the same genes affect the studied trait in males and females in each population. The analysis of r_{mf}s revealed two different patterns, r_{mf} was not significantly different from 0 in

Table 2 Results of the ANOVA testing for differences in mean desiccation resistance (LT50) among lines (within populations), populations and desiccation treatments and between sexes of *D. buzzatii*

	SS	df	MS	F	*p*
Intercept	930,412.8	1	930,412.8	877.19	0.001
Population	12,649.1	3	4,216.4	3.97	0.015
Line (population)	38,184.3	36	1,060.7	2.70	0.001
Sex	2,246.8	1	2,246.8	13.50	0.001
L (P) * S	5,989.3	36	166.4	6.59	0.001
Treatment	71,125.1	2	35,562.6	141.69	0.001
L (P) * T	18,071.0	72	251.0	9.95	0.001
P * S	1,152.9	3	384.3	2.31	0.092
P * T	1,096.7	6	182.8	0.72	0.628
S * T	75.3	2	37.6	1.49	0.225
P * S * T	106.4	6	17.7	0.70	0.647
Error	26,016.8	1,032	25.2		

df degrees of freedom, *SS* sums of squares, *MS* mean squares

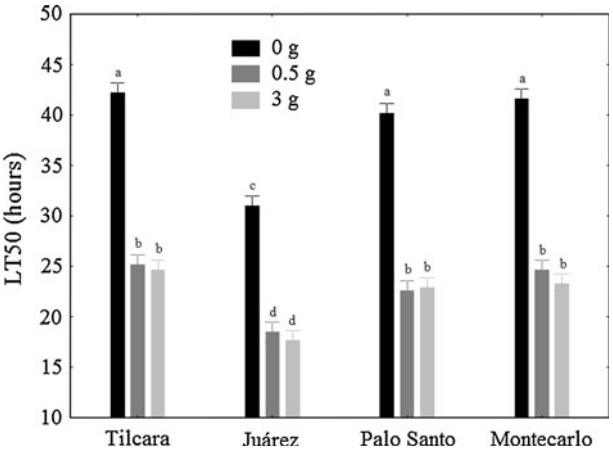

Fig. 1 Effects of treatments on desiccation resistance (LT50) in each population ($F = 0.73$; $df = 6$; $p = 0.63$). *Vertical bars* denote 0.95 confidence intervals. Means with the same letter are not significantly different

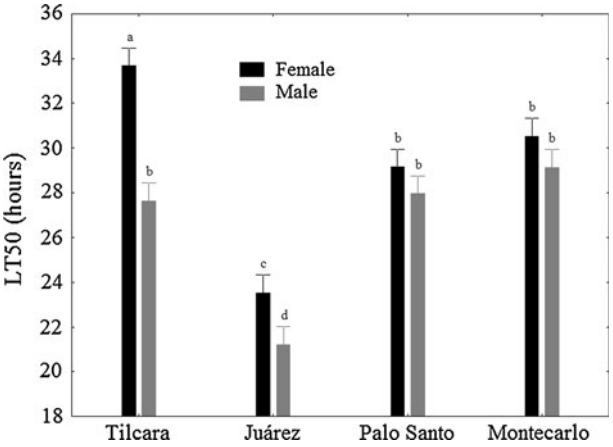

Fig. 2 Effects of sex on desiccation resistance (LT50) in each population ($F = 2.31$; $df = 3$; $p = 0.09$). *Vertical bars* denote 0.95 confidence intervals. Means with the same letter are not significantly different

Tilcara, whereas it was significantly higher than 0 and lower than 1 in the remaining populations (Table 3). These results are consistent with the trends detected in the ANOVAs and the regression analysis.

We also measured thorax length as a surrogate of body size in the same set of lines studied for desiccation resistance. The ANOVA showed that only differences between sexes were significant indicating that females are significantly larger than males (Tukey posthoc test $p < 0.05$). At variance with desiccation resistance, sexual dimorphism for thorax length did not significantly vary across populations as indicated by the non-significant population-by-sex interaction (Fig. 4).

Finally, we analyzed desiccation resistance in the progeny from crosses between lines to assess the genetic components through linear regressions of LT50 on genotype.

Table 3 Results of the ANOVAs testing for differences in desiccation resistance between sexes and among lines, and relative contribution of each source of variation to total phenotypic variation in each population of *Drosophila buzzatii*

	Tilcara	Juárez	Palo Santo	Montecarlo
Sex	*	n.s.	n.s.	n.s.
Line	4.42	45.77**	15.20*	30.15**
Line * sex	13.91*	6.93***	6.34***	11.67***
Error	15.42	14.36	23.75	18.63
rmf[a]	0.24 (0.32)	0.79 (0.20)	0.64 (0.25)	0.68 (0.24)

[a] Cross-sex genetic correlation coefficient (SE)

Significance level: *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

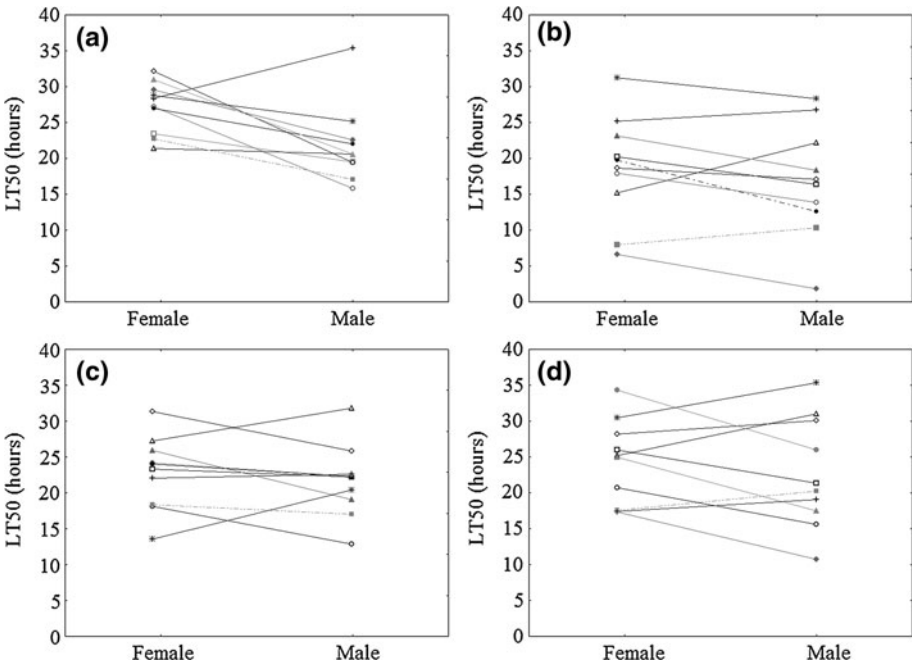

Fig. 3 Desiccation resistance in males and females in lines derived from **a** Tilcara, **b** Juarez, **c** Palo Santo and **d** Montecarlo

Regressions were significant in seven out of ten crosses. The proportion of among-genotype variance explained by the regression was greater than 40 % in four crosses, suggesting that additivity is the most likely effect of the genetic factors underlying desiccation resistance, whereas in the remaining three significant crosses the proportion of among-genotype variance varied between 12 and 19 % suggesting dominant effects. Nevertheless, epistatic interactions cannot be ruled out (Table 4, Appendix). A posteriori contrasts, performed in crosses in which regression analyses yielded significant results, showed that in some crosses F1 genotypes expressed intermediate phenotypes, significantly different from both parental lines, while in other crosses the mean phenotype of hybrids was close to one of the parental lines, pointing to deviations from additivity (Table 5, Appendix).

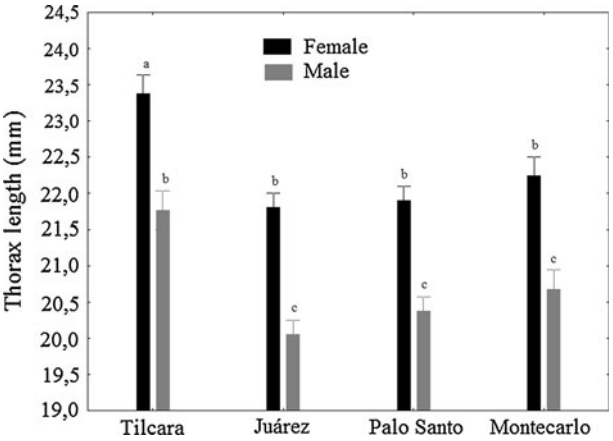

Fig. 4 Size differences between sexes in each population ($F = 0.46$; $df = 3$; $p = 0.71$). *Vertical bars* denote 0.95 confidence intervals. Means with the same letter are not significantly different

Discussion

A main avenue in evolutionary biology is the search for patterns of geographic variation that, at the species level, may represent the hallmark of natural selection. A priori, we expected to find a less intense effect of desiccation treatments on flies from desert areas as a consequence of adaptation to this type of environment. However, at first sight our results do not give support to this hypothesis since neither flies from Juárez, located in the arid extreme of North Eastern Argentina, nor flies from Tilcara, located in a very arid valley in North Western Argentina, exhibited increased resistance to experimental desiccation. Actually, mean desiccation resistance of Tilcara flies did not differ significantly (but see below) and Juárez flies exhibited decreased survival when compared to populations inhabiting mesic (Palo Santo) or even humid areas (Montecarlo).

However, the picture that arose after evaluating the possible correlates of among-population differentiation in desiccation resistance with environmental climatic variables is fairly complex, since males and females exhibited quite different patterns. On the one hand, regression analyses showed that male desiccation resistance is not associated with any of the environmental variables considered. On the other hand, several climatic variables were correlated with among-population variation in female desiccation resistance. First, our results suggest a positive relationship between female LT50 with altitude, a trend that is in agreement with our *a priori* expectations since atmospheric pressure decreases, and consequently water evaporation increases, with altitude. In fact, females from Tilcara, where water loss through evaporation is intense, were the most resistant to desiccation as compared to flies derived from the other collecting sites. However, it is worth noting that Tilcara is the only sampling site located above 2,000 m above sea level (asl) sampled for this study, the other collecting sites lie below 1,000 m asl. Also, maximal temperatures are highly correlated with mean annual temperature and saturation vapour pressure (SVP), and these variables are negatively related to females' resistance, in agreement with expectations. Indeed, increases in temperature and rainfall are geographically coincident and, as a result, relative humidity tends to increase as we move eastwards, supporting an East to West gradient in terms of water stress.

Further regression analysis revealed that among-population variation in sexual dimorphism might be partially accounted for by its positive correlation with altitude. In addition,

rainfall, minimal temperature and aridity index were negatively associated to female: male ratio, suggesting that sexual dimorphism is more apparent in populations facing the most pronounced desert conditions. We acknowledge that a more comprehensive sampling strategy is needed to understand the relationship between desiccation resistance and environmental variables, since our study may be capturing only a portion of the biogeographic pattern, instead of focusing on detailed biological variability. Nevertheless, our results jointly suggest that the patterns of clinal variation detected in females and for the sexual dimorphism may be considered as indicative of strong selective forces shaping variation in desiccation resistance.

Thus, our study adds to a long lasting issue concerning the determinants of patterns of natural variation in desiccation resistance. Previous studies either gave support or rejected adaptive explanations, depending on the species (Karan et al. 1998), clines (Da Lage et al. 1990; Hoffmann et al. 2001) and populations compared (Nevo et al. 1998; Matzkin et al. 2007; Gilchrist et al. 2008). Overall, research on desiccation resistance has led to the general conclusion that *Drosophila* species vary widely in their ability to respond to seasonal or spatial changes in water stress (Hoffmann and Harshman 1999).

Demographic, historical or ecological factors can account for our observed pattern, such as geographic barriers or differences among the host cacti that flies use as breeding and feeding resources. Concerning history and demography, it is worth mentioning that *D. buzzatii* has dispersed passively and rapidly to new regions following its preferred host plants. Such rapid expansion implied bottlenecks that resulted in loss of genetic variation (Fontdevila 1989; Piccinali et al. 2007) and/or non-adaptive evolution (Fontdevila 1989; Hasson et al. 1995; Rossi et al. 1996). Regarding ecological factors, *D. buzzatii* breeds and feeds mainly on necrotic tissues of several species of the genus *Opuntia* that provide a relatively constant, homogeneous and benign environment to the developing larvae (Hasson et al. 2009). The duration of *Opuntia* necrotic pockets as suitable breeding sites depends on cladode size, water content, chemical composition and also climate variables (Hasson et al. 1992).

In this study, we found that the populations analyzed are not only differentiated but also harbor substantial amounts of genetic variation in their ability to survive under desiccating conditions. In fact, the significant line effect may be construed as evidence that an important fraction of variation in desiccation resistance has a genetic basis. However, differences among lines were context-dependent as indicated by the line-by-treatment (which under our experimental design may be interpreted as a genotype-by-environment interaction -GEI-) and line-by-sex (i.e. genotype-by-sex interaction -GSI-) interactions. Regarding desiccant treatments, a preliminary study (Sassi and Hasson 2008) reported significant differences between 0.5 g and 3 g treatments in a reduced set of lines, indicating that lines were differentially affected by the intensity of desiccation treatments. Nevertheless, although differences between desiccation treatments were not significant in the present study, this result does not imply a null contribution of the treatment factor to total variation in desiccation resistance, since the treatment-by-line-interaction is still significant. In any case, it is important to recall that our present results are based on a substantially larger number of populations and lines.

GEIs are usually invoked as possible explanations for the maintenance of quantitative variation, whereas significant GSIs may be considered indicative of sex-specific responses (Mackay et al. 2009). We also detected a significant sexual dimorphism for the trait under study, with females being more resistant than males, however, the magnitude of sexual dimorphism varied among lines (genotypes) and populations. Furthermore, the analysis of across-sex genetic correlations (r_{mf}) denotes a high degree of independence between the

sets of genes affecting variation in desiccation resistance in males and females in the population facing the highest aridity (Tilcara), suggesting potential independent evolution of sexes. In contrast, r_{mf} values were significantly different from 0 and 1 (but close to 1) in the remaining populations, indicating that the genetic architecture of desiccation resistance involves partly overlapping genes across sexes. In addition, the results of crosses between lines exhibiting extreme phenotypic values confirm the involvement of genetic factors underlying desiccation resistance and that the differences between lines are explained by complex patterns of additive, dominance and epistatic interactions.

Artificial selection experiments have demonstrated that desiccation resistance is correlated with several biological variables such as starvation resistance, heat resistance, body mass, age, life span, metabolic rate and activity levels (Hoffmann and Parsons 1993; Gibbs et al. 1997; Hercus and Hoffmann 1999; Nghiem et al. 2000). Thus, apparent non-adaptive responses of desiccation resistance might be attributed to other selective pressures such as food shortage, resource characteristics and extreme thermal variation acting upon correlated traits, rather than to direct selection. Particularly in *D. buzzatii*, the response to extreme temperatures, that involves resistance to heat stress and heat shock protein expression, correlates with desiccation resistance (Sørensen et al. 2005). Thus, the occurrence of such trait correlations could explain the relatively and unexpected high resistance in Eastern (Palo Santo and Montecarlo) populations since, despite the high relative humidity, they experience slight temperature differences with respect to Western (Tilcara and Juárez) populations (Table 1). In addition, the cited authors compared populations throughout an altitudinal gradient and reported a significant effect of population and sex on desiccation resistance, in agreement with our results.

Dehydration tolerance has been proposed as a likely mechanism for desiccation resistance, although rarely confirmed (Gibbs and Matzkin 2001). Alternatively, a decreased rate of water loss has also been suggested as a possible mechanism, whereas changes in carbohydrate levels and wet weight are occasionally associated with desiccation resistance (Hoffmann and Harshman 1999; Gibbs et al. 2003; Marron et al. 2003; Archer et al. 2007). Other studies have shown that selection for increased desiccation tolerance may result in correlated responses of carbohydrate storage, haemolymph volume and body weight (Folk et al. 2001; Folk and Bradley 2005), accumulation of metabolic resources (Djawdan et al. 1997), and carbohydrate metabolism and energy consumption (Marron et al. 2003). Overall, these studies suggest that increased desiccation resistance can arise through multiple evolutionary pathways.

In this context, our geographic survey showing that the magnitude of sexual dimorphism varies across populations and correlates with an aridity gradient, constituting a biogeographic pattern, may help understand the mechanisms underlying desiccation resistance in *D. buzzatii*. Interestingly, sex-specific patterns in desiccation resistance and resource acquisition have been reported in lines selected for desiccation resistance in *D. melanogaster* (Chippindale et al. 1998; Archer et al. 2007). Moreover, it has been proposed that water stress triggers contrasting evolutionary responses in females and males and that selection for increased desiccation tolerance may lead to strong selection for early maturation and mating in males and for resource acquisition and survival in females (Kwan et al. 2008). Such sex-specific responses may lead to a reinforcement of the usual sexual dimorphism observed under more benign environments and point to some kind of intersexual antagonism in the evolution of stress resistance.

Thus, our results may be considered as evidence against a low rate of water loss as the mechanism behind the pattern observed in *D. buzzatii*. Indeed, water loss is influenced by body size (Gibbs et al. 1997), but even though all studied populations showed similar

sexual dimorphism in body size, desiccation resistance was sexually dimorphic only in those from arid localities. Therefore, we propose that accumulation of glycogen reserves (glycogen can bind 3–5 times its weight in water -Schmidt-Nielsen 1997) may be a more plausible mechanism underlying desiccation resistance in *D. buzzatii* since it is compatible with the biogeographic trend detected for sexual dimorphism, and with sex-specific responses to water stress (Chippindale et al. 1998; Matzkin et al. 2007; Kwan et al. 2008).

Sexual dimorphism in starvation resistance has been ascribed to differences in the basic physiological processes underlying allocation of limiting energy reserves between survival and reproduction (Goenaga et al. 2010 and references therein). Similarly, since desiccation resistance is expensive as well (Marron et al. 2003), energetic trade-offs plus sex-specific responses and reproductive strategies could take place under water stress. A variable pattern of resource accumulation could support a plastic strategy at the species level. The fact that desiccation resistance presents a sex-specific genetic architecture in Tilcara explains the marked sexual dimorphism in this population. In turn, the overlapping genetic basis of desiccation resistance in males and females in the other populations would explain sexual dimorphism only under water stress conditions (Juárez), and its erosion in populations that colonized mesic and humid regions. However, since our study only provides indirect evidence, future studies measuring glycogen and water content in natural populations, and comparing reproductive-related traits between sexes along the same aridity gradient, are necessary to fully understand desiccation resistance in *D. buzzatii*.

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Appendix

See Tables 4 and 5.

Table 4 Results of linear regressions of desiccation resistance on genotype in ten crosses between lines showing extreme phenotypic values

Hybrid	R (beta)	R^2	Adjusted R^2	F (1,58)	SE	SE estimate	*p*
Til10 × Jua23	0.40	0.16	0.15	11.22	0.12	12.13	0.01
Til10 × Jua33	0.71	0.51	0.50	61.11	0.09	11.21	0.01
Til10 × Til16	0.03	0.01	–	0.05	0.13	13.77	0.89
Til10 × Jua27	0.04	0.01	–	0.10	0.13	13.85	0.75
Til16 × Jua23	0.37	0.14	0.12	9.46	0.12	12.19	0.01
Til16 × Jua33	0.66	0.44	0.43	45.42	0.09	12.53	0.01
Til16 × Jua27	0.07	0.01	–	0.36	0.13	12.61	0.55
Jua23 × Jua33	0.64	0.41	0.41	41.32	0.10	7.31	0.01
Jua23 × Jua27	0.46	0.21	0.19	15.42	0.11	11.48	0.01
Jua33 × Jua27	0.74	0.56	0.55	73.00	0.08	10.77	0.01

Table 5 Results of 'a posteriori' comparisons in crosses between pairs of lines in which regression analyses yielded significant results (see text for details)

Lines	Til10	Til16	Jua23	Jua33	Jua27
Til10 × Jua23	***		ns		
Til10 × Jua33	ns			***	
Til16 × Jua23		*	ns		
Til16 × Jua33		ns		***	
Jua23 × Jua33			**	**	
Jua23 × Jua27			ns	***	
Jua33 × Jua27			***	**	

Significance level: $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$

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