Austral ECOLOGY A Journal of ecology in the Southern Hemisphere

Austral Ecology (2008) **33**, 663–671

A comparative study of competitive ability between two cactophilic species in their natural hosts

VICTORIA WERENKRAUT,[1,2] ESTEBAN HASSON,[1] LUCIANA OKLANDER[1,3] AND JUAN J. FANARA[1]*

[1]*Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria Pab. II. (C1428EHA) Buenos Aires, Argentina (Email: jjfanara@ege.fcen.uba.ar),* [2]*Laboratorio Ecotono, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Bariloche, and* [3]*Museo Argentino de Ciencias Naturales e Instituto de Investigaciones en Ciencias Naturales, Buenos Aires, Argentina*

Abstract Competition is a major aspect of the ecology of insect communities exploiting ephemeral and fragmented resources. We analysed the effect of intraspecific (single species culture) and interspecific (mixed species culture) competition on larval viability, developmental time and wing length in the cactophilic *Drosophila buzzatii* and *Drosophila koepferae* (Diptera: Drosophilidae) reared in cultured media prepared with fermenting tissues of three common natural cactus hosts in nature at different densities. Our results show that all traits measured were affected by both intra- and interspecifc competition, although the effect of competition depended on the *Drosophila* species and the rearing cactus. In fact, flies tended to have a lower viability, shorter wing size and longer developmental time as a function of increasing density in single species culture in both *D. buzzatii* and *D. koepferae* (intraespecific competition). Besides, the performance of both species was seriously affected (shorter body size, slower developmental times, lower viability) by the presence of heterospecific competitors except in the case of *D. koepferae* reared in its primary host plant, *Trichocereus terschekii*. We also show that *D. koepferae* successfully utilized *Opuntia quimilo,* which is absent in most parts of its distribution range. We discuss the roles of intra- and interspecific competition as determinants of the relative abundance of these two species in the arid zones of Southern South America.

Key words: colonization, habitat selection, competition, *Drosophila* cactophilic, host plant.

INTRODUCTION

Many of the major events in the diversification of life can be traced back to the appearance of novel species interactions (Margulis & Fester 1991; Maynard Smith & Szathmary 1995). Moreover, studies of species interactions within natural communities suggest that interactions between a pair or group of species can differ greatly in outcome across landscapes (Thompson 1999; Thompson & Cunningham 2002). Competition is an interaction between organisms or species, brought about by a shared requirement for a resource in limited supply. As a consequence of competition, some of the individuals involved exhibit reductions in the survivorship, growth and/or reproductive success (Cockburn 1991; Santos *et al.* 1992a; Joshi *et al.* 1996; James & Partridge 1998).

Different models have been proposed to explain how ecologically similar species can coexist avoiding the effects of competition. According to the aggregation model of coexistence two species may coexist indefinitely if they exhibit aggregated but independent distributions among patches (Shorrocks & Rosewell 1987; Rosewell *et al.* 1990). The habitat selection model has also been invoked (Rosenzweig 1991; Morris 1999) wherein ecological specialization and niche expansion can be the result of trade-offs in performance across different environments (Rausher 1984; Diehl & Bush 1989; Hopf *et al.* 1993; Rainey & Travisano 1998).

Most *Drosophila* species live in conditions characterized by limited and ephemeral resources in nature (Etges & Heed 1987; Robertson 1987; Thomas 1993; Quezada Diaz *et al.* 1997), which promote competition. Competition is expected to mainly affect larval performance as the larval stage may be the most important phase of resource limitation (Nunney 1990; Santos *et al.* 1992a; Roper *et al.* 1996; Quezada Diaz *et al.* 1997; Shiotsugu *et al.* 1997). However, the available evidence indicates that the consequences of competition are not limited to the larval stages. For instance, competition for resources during larval life may affect adult body size (Robertson 1987; Etges &

*Corresponding author.

Accepted for publication August 2007.

doi:10.1111/j.1442-9993.2008.01833.x

Heed 1987; Quezada Diaz *et al.* 1997), which is usually correlated with reduced fecundity and shortened life span (Santos *et al.* 1992b; Rodriguez *et al.* 1999).

Cactophilic species of the genus *Drosophila* belonging to the repleta group are an excellent model system for the study of competition and adaptation because of their well-known ecology (Powell 1997). *Drosophila buzzatii* and *D. koepferae* are two cactophilic sibling species that belong to the *D. buzzatii* complex of the repleta group (Wasserman 1992; Ruiz & Wasserman 1993). The former has a subcosmopolitan distribution (Barker & Starmer 1982; Fontdevila 1989; Hasson *et al.* 1992), whereas the latter is restricted to the deserts of Southern South America where both species have partially overlapping distributions (Fontdevila *et al.* 1988; Hasson *et al.* 1992; Piccinali *et al.* 2004). Studies in natural populations from Argentina have shown that *D. buzzatii* emerges primarily from the necrotic cladodes of prickly pears (genus *Opuntia* as *O. quimilo*, *O. ficus-indica* and *O. sulphurea*), and secondarily from columnar cacti (*Trichocereus terschekii* and *T. candicans,* for example), whereas the reverse is true for *D. koepferae* (Fontdevila *et al.* 1988; Hasson *et al.* 1992; Fanara *et al.* 1999). However, they are not differentially attracted to the necroses of their main host plants (Fanara *et al.* 1999). Three explanations have been offered to elucidate the differences in the proportions of both species attracted to, and emerged from, columnars and prickly pears: host specific fitness, oviposition preferences and/or interspecific competition. Laboratory studies have shown that both species maximize larval viability (LV), developmental time (DT) and body size in their main host plant, i.e. *D. buzzatii* in *Opuntia* cacti and *D. koepferae* in columnar cacti, though these differences cannot explain the observations in natural populations (Fanara *et al.* 1999). Concerning oviposition preferences, *D. buzzatii* and *D. koepferae* differ sharply in their acceptance of both hosts (Fanara *et al.* 1999). Specifically, *D. buzzatii* lays more eggs on rotting materials of *Opuntia* while *D. koepferae* prefers columnar cacti as an oviposition site (Fanara & Hasson 2001). Finally, studies addressing the effect of interspecific competition in this pair of sibling species that exhibit a certain degree of niche overlap are lacking, although there was an attempt to fill this gap. In fact, Fanara *et al.* (2004) showed that the presence of one species affected the performance of the other by studying the performance of both species in single and mixed species cultures, in which the proportion of each species in the eggs seeded in the cactus media was largely unknown.

In the present paper we examine the effects of intra- and interspecific competition on the general performance of *D. buzzatii* and *D. koepferae* in vials started with varying proportions of both species and different densities in media prepared with fermenting tissues of three alternative host plants.

MATERIALS AND METHODS

Flies analysed in the present study were collected in the locality of Ruinas de Quilmes (26°27′22″S, 66°02′46″W, Tucumán Province, Argentina), which lies in the arid Monte phytogeographical province (see Fanara *et al.* 1999 for further details). In this locality, there are two different host cacti that serve as breeding and feeding resources for the flies: *O. sulphurea* and *T. terschekii*. As we describe below, we also included another prickly pear species, *O. quimilo,* which is not present in the site of collection, but is the most common host plant in the Chaco phytogeographical province, where *D. buzzatii* is the dominant *Drosophila* species and *D. koepferae* is at very low density (Hasson *et al.* 1992).

Flies were collected by means of net sweeping on fermented banana baits and sorted by sex. Isofemale lines were founded in vials containing 5 mL of David's (1962) killed yeast laboratory medium and identified to species by the inspection of the genitalia of one male progeny (Vilela 1983; Fontdevila *et al.* 1988). Twenty-two *D. koepferae* and 20 *D. buzzatii* isofemale lines were used in the foundation of two outbreed stocks, one of each species. Isofemale lines were reared individually in identical conditions for three generations in bottles with 30 mL of laboratory medium and never exposed to the medium prepared with rotting cactus. We also collected fresh cacti and juice exudates from pieces of naturally occurring rotting cacti. Fresh and rotting *O. quimilo* were collected in the locality of Río Hondo (27°30′37″S, 64°51′13″W, Santiago del Estero Province, Argentina) that belongs to the Chaco phytogeographical province (for a description see Fanara *et al.* 1996). Pieces of fresh cactus were stored at –20°C and the fermenting juice of each cactus maintained in the laboratory by adding 10 g of fresh cactus every 2 weeks until the onset of the experiments. For the preparation of the cactus media, pieces of cactus of a given species were mixed in a blender and 5 mL poured into glass vials and autoclaved. After cooling, each vial was inoculated with 0.1 mL of the fermenting juice and incubated at 25°C for 24 h.

Large quantities of first instar larvae of each species were obtained by placing batches of 100 pairs of sexually mature flies into egg-collecting chambers (for a description see Fanara *et al.* 1999). Eight chambers were set up for each combination of *Drosophila* (*D. buzzatii* and *D. koepferae*) and cactus species (*O. sulphurea*, *O. quimilo* and *T. terschekii*). Egg-laying medium was poured into Petri dishes (8 cm of diameter) and the fermenting juice of the corresponding

doi:10.1111/j.1442-9993.2008.01833.x

rotting cactus species was spread onto the agar surface to stimulate oviposition. Chambers were prepared in the morning and 8 h later all flies were removed. Dishes were incubated for an additional 24 h at 25°C to allow egg hatching. Batches of 40 first instar larvae were collected from the dishes and seeded in vials containing the same cactus medium used to stimulate oviposition.

Two different types of vials were set up: single (intraspecific competition) and mixed (interspecific competition) species cultures. In single species cultures, 40, 80 and 120 first instar larvae (low, medium and high density, respectively) of one species (100% *D. koepferae* or 100% *D. buzzatii*) were seeded in vials containing cactus media. In mixed species cultures interspecific competition was studied by means of substitution experiments in the three cactus media. In these vials, both *Drosophila* species were initially present in the same proportions but the total number of larvae varied according to the density (40, 80 or 120 larvae per vial). As an example, 40 larvae of each species were seeded in mixed species vials at medium density (80 larvae per vial). We ran five replicated vials for each combination of *Drosophila* species, type of culture, cactus and density, making a total of 10 800 first instar larvae seeded in 135 vials. All experiments were conducted at 25°C and at a photoperiod of 14:10 light : dark.

Emerging adults were collected daily at 9 AM and sorted by sex. LV, DT and body size were scored only in males as females of both species are morphologically indistinguishable. We determined that this procedure was valid as the proportion of males and females emerged from single species cultures in all combinations of *Drosophila* species, cactus and density (data not shown) did not depart from the expected 1:1, indicating that survival was independent of sex. Thus, LV was measured in each vial, as the proportion of males of each *Drosophila* species emerged relative to the number of first instar larvae of each *Drosophila* species initially seeded in the vials divided by two. For instance, LV in *D. buzzatii* reared in mixed species culture at medium density (80 larvae) was calculated as the number of emerging *D. buzzatii* males relative to 20 as 40 larvae (20 male and 20 female) were seeded of each *Drosophila* species in each vial. DT was estimated as the time elapsed since first instar larvae were transferred to the vials until adult emergence. We also measured wing length (WL) as an estimation of body size. Three to five *D. buzzatii* and *D. koepferae* males emerged in each vial were randomly chosen and the right wing removed. WL was measured as the distance between the intersection of the second and the third veins and the distal end of the latter using a binocular microscope fitted with an ocular micrometer.

Larval viability, DT and WL were analysed by means of ANOVAs in which each vial (replicate) was considered the experimental unit, with *Drosophila* species (*D. buzzatii vs. D. koepferae*), type of culture: (single *vs.* mixed culture), cactus (*T. ter*schekii *vs. O. quimilo vs. O. sulphurea*) and density (low *vs.* medium *vs.* high), all fixed factors. Thus, the mean values of DT and WL, averaged across all individuals scored, in each replicate were included in the analysis. Our ANOVA designs contained a large number of interactions that allowed us to investigate different issues related to ecological factors that affect the performance of both *Drosophila* species. First, the interaction *Drosophila* species by type of culture tested whether species performed differently alone (intraspecific competition) or in competition (interspecific competition). Second, the interaction *Drosophila* species by type of culture by cactus tested whether intra- and interspecific competition affected differentially *D. buzzatii* and/or *D. koepferae* in the different rearing media. Finally, the interaction *Drosophila* species by cactus tested the differential effect of the rearing media on each *Drosophila* species.

Tukey's tests were used for *a posteriori* comparisons when necessary (Sokal & Rohlf 1985). Prior to the ANOVAs, LV data (expressed as proportions) were angularly transformed and, DT and WL were log transformed. All statistical analyses were performed using the GLM procedure implemented in the STATISTICA software package (StatSoft, Tulsa, OK).

RESULTS

Estimates of viability in single species cultures of *D. koepferae* were relatively high at all densities (78%, 79.4% and 64.2% at low, medium and high density, respectively). Viability in *D. buzzatii* was also relatively high at low (79%) and medium densities (62.5%), though it substantially decreased at high density (37.5%).

Our results also show (Table 1) that, on average, *D. koepferae* had larger WL and longer DT than *D. buzzatii*, and that flies raised in mixed species cultures tended to be smaller and to develop faster than in single species culture. Likewise, lower LV, smaller WL and longer DT were recorded at increasing densities indicating an effect of crowding on flies' performance. In addition, flies reared in *O. sulphurea* exhibited higher survival, larger WL and shorter DT, whereas flies emerged in *T. terschekii* vials showed, in average, a poorer performance for all traits analysed when compared with flies reared in *O. quimilo* and *O. sulphurea*. However, it is important to note that the significant interactions involving the main factors detected in the ANOVAs (Table 2) preclude the direct interpretation of the differences observed between species, cactus media and type of culture and among densities (Table 2). Thus we explore the interactions to test our inquiry. The *Drosophila* species by cactus interaction

doi:10.1111/j.1442-9993.2008.01833.x

Table 1. Mean and standard deviation (between parentheses) of larval viability (in percentage of survival), developmental time (in days) and wing length (in millimetre) for *Drosophila buzzatii* and *D. koepferae* reared in single and mixed species culture raised at low, medium and high density (40, 80 and 120 larvae per vial, respectively) in vials prepared with *Opuntia quimilo, O. sulphurea* and *Trichocereus terschekii*

Factor	Level	*n*	Larval viability	Developmental time	Wing length
Drosophila species	*D. buzzatii*	90	64.04 (2.48)	12.49 (1.62)	1.87 (0.08)
	D. koepferae	90	59.68 (2.22)	13.15 (1.57)	2.01 (0.12)
Type of culture	Single	90	59.94 (1.77)	13.31 (1.46)	1.96 (0.12)
	Mixed	90	63.78 (2.82)	12.34 (1.64)	1.92 (0.13)
Density	Low	60	69.58 (2.46)	11.67 (0.94)	2.00 (0.11)
	Medium	60	60.58 (2.54)	12.70 (1.56)	1.94 (0.12)
	High	60	55.42 (1.80)	14.10 (1.29)	1.88 (0.13)
Cactus	*O. quimilo*	60	61.06 (2.31)	13.15 (1.41)	1.93 (0.12)
	O. sulphurea	60	68.75 (1.94)	11.87 (1.18)	2.02 (0.10)
	T. terschekii	60	55.78 (2.61)	13.45 (1.79)	1.88 (0.12)

n, sample size.

Table 2. Analysis of variance testing for differences in larval viability (LV), developmental time (DT) and wing length (WL) in *Drosophila buzzatii* and *D. koepferae* reared in three different host cacti (*Opuntia sulphurea*, *O. quimilo* and *Trichocereus terschekii*), reared in mixed and single species culture at three different densities (40, 80 and 120 larvae per vial)

		LV		DT		WL	
	d.f.	SS	*F*	SS × 10^{-2}	*F*	SS × 10^{-2}	*F*
Drosophila species (A)	1	0.039	2.71	2.39	52.41*	4.16	273.40*
Type of culture (B)	1	0.014	0.33	5.45	135.67*	0.41	26.77*
Density (C)	2	0.281	9.51*	19.31	240.30*	2.54	83.60*
Cactus (D)	2	0.285	9.68*	9.20	114.50*	3.11	102.39*
A × B	1	0.278	18.83*	0.77	19.20*	0.09	5.94**
A × C	2	0.061	2.06	0.28	3.54**	1.35	4.26**
B × C	2	0.077	2.61	0.53	6.59***	0.08	3.10**
A × D	2	0.071	2.42	0.80	9.92*	0.23	7.69*
B × D	2	0.221	7.52*	0.64	7.98*	0.56	18.45*
C × D	4	0.230	3.89***	2.92	18.14*	0.71	11.65*
A × B × C	2	0.060	2.04	0.03	0.36	0.01	0.18
A × B × D	2	0.295	10.02*	0.79	9.84*	0.12	3.42**
A × C × D	4	0.128	2.17	0.62	3.85***	0.05	0.78
B × C × D	4	0.056	0.95	1.29	8.01*	0.11	1.85
A × B × C × D	4	0.114	1.939	1.04	6.50*	0.02	0.38
Error	144	2.124	–	5.79	–	2.19	–

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

was significant for DT and WL (Table 2), indicating that these traits in *D. buzzatii* and/or *D. koepferae* depended on the rearing media used. In fact, DT and WL differences between *Drosophila* species were smaller in *T. terschekii* than in both *Opuntia* (not shown). The significant *Drosophila* species by density interaction detected in the ANOVAs for DT and WL suggest that intraspecific competition differentially affected these traits in *D. buzzatii* and *D. koepferae* (Table 2). However, the trends observed for each trait were different. Indeed, DT differences between *D. buzzatii* (11.58, 12.24 and 13.81 days at low, medium and high density, respectively) and *D. koepferae* (11.96, 13.44 and 14.37 days at low, medium and high density, respectively) were largest at intermediate (80 larvae per density) density, while differences for WL between *D. buzzatii* (1.792, 1.781 and 1.772 mm at low, medium and high density, respectively) and *D. koepferae* (1.828, 1.817 and 1.792 mm at low, medium and high density, respectively) were significantly smaller at low density (40 larvae per vial) than at intermediate and high densities (data not shown).

The *Drosophila* species by type of culture interaction was also significant in the ANOVAs for LV, WL and DT, although the pattern observed varied among traits (Fig. 1). The trends observed for WL and DT were similar across species. In effect, *D. buzzatii* and *D. koepferae* had smaller wings and developed more

doi:10.1111/j.1442-9993.2008.01833.x

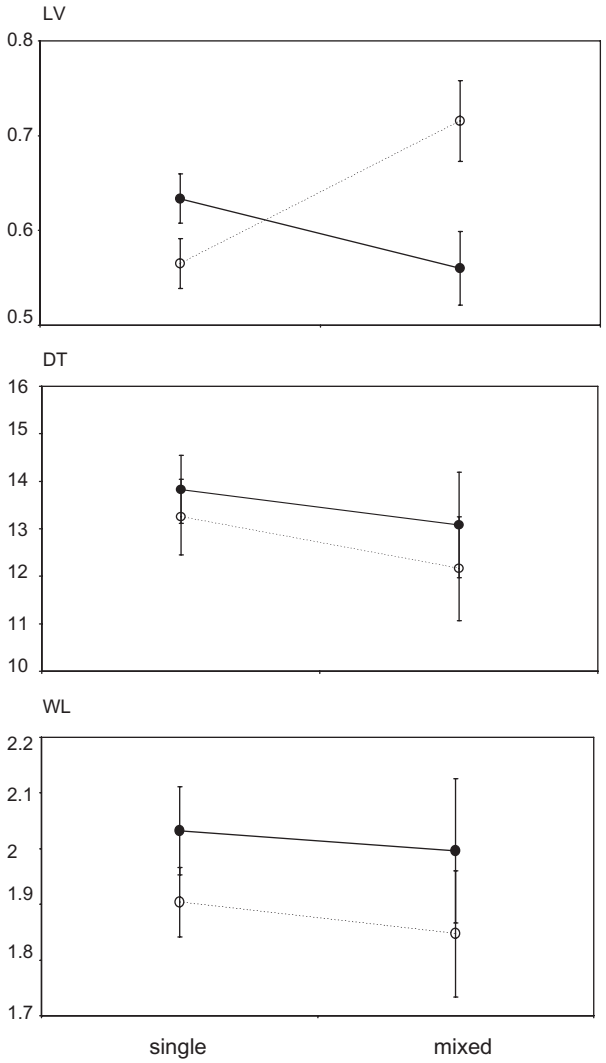

Fig. 1. Mean and standard deviation of larval viability in percentage of survival (LV), developmental time in days (DT) and wing length in millimetre (WL) of *Drosophila buzzatii* (open circle) and *D. koepferae* (filled circle) reared in single and mixed species culture.

slowly in mixed than in single species cultures (Fig. 1; Turkey's test, $P < 0.05$ for both *Drosophila* species), although differences were larger in *D. buzzatii.* Regarding LV the effect of the type of culture was more noticeable in *D. buzzatii* than in *D. koepferae*, as survival in the former significantly decreased in single relative to mixed species cultures (Fig. 1; Turkey's test, $P < 0.01$). Besides, the strength of the effect of interspecific competition on WL and DT differed across densities as suggested by the significant type of culture by density interaction (Table 2). Actually, the effect of interspecific competition was strongest at high density for both traits (data not shown).

The results described above suggest that both *Drosophila* species are differentially affected by intra- and interspecific competition. However, according to the significant interaction *Drosophila* species by type of culture by cactus the effect of the type of culture on LV, DT and WL also depended on the cactus medium (Table 2). Neither *D. buzzatii* nor *D. koepferae* showed significant differences in LV when reared in mixed *versus* single species cultures in *O. sulphurea*. In contrast, LV differences between mixed and single species cultures were significant in both *D. buzzatii* and *D. koepferae* in *T. terschekii*, though species' responses differed between cactus hosts (Fig. 2). *Drosophila koepferae* had a significantly lower LV in mixed species cultures raised in *T. terschekii* (Turkey's test, $P < 0.01$), while *D. buzzatii* showed a significantly lower LV in single species cultures (Turkey's test, $P < 0.01$). In *O. quimilo*, only *D. buzzatii* showed a significant lower viability in single species cultures (Turkey's test, $P < 0.01$). Regarding DT, both species developed faster in mixed than in single species cultures (Fig. 2; Turkey's test, $P < 0.01$) with the exceptions of *D. koepferae* grown in *O. quimilo* and *D. buzatii* in *O. sulphurea*, in which differences between types of culture were not significant. Finally, flies of both *D. buzzatii* and *D. koepferae* species, emerged from mixed species vials had, on average, a smaller WL than in single species cultures in both *O. quimilo* and *O. sulphurea* (Fig. 2; Turkey's test, $P < 0.01$) although this pattern was different in *D. koepferae* reared in *T. terschekii* owing to this species exhibited larger WL in mixed than in single species cultures (Fig. 2; Turkey's test, $P < 0.01$).

DISCUSSION

The present study showed that LV, DT and WL are affected by both intra- and interspecifc competition in *D. buzzatii* and *D. koepferae*. Although intraspecific aggregation can be adaptive under certain circumstances (Rohlfs & Hoffmeister 2004; Sanders *et al.* 2005), crowding can cause a severe decline in overall performance (Santos *et al.* 1997; James & Partridge 1998; Baldal *et al.* 2005). The presence of conspecific and heterospecific competitors may constitute a limiting factor for the growing larvae because it can affect the quality and/or quantity of food supply. Thus, larvae would need to spend more time feeding until achieving a certain critical size to pupate (Bakker 1961; Prasad *et al.* 2001). In this sense, our results show that body size and DT were decreasing and increasing functions of density, respectively, suggesting that both *D. buzzatii* and *D. koepferae* suffered a detrimental effect at higher densities. Similarly, LV decreased in crowded conditions.

Likewise, the observed differences in performance between flies grown in columnar and *Opuntia* cacti can also be explained by differences in food quality between hosts, and are in line with available evidence

doi:10.1111/j.1442-9993.2008.01833.x

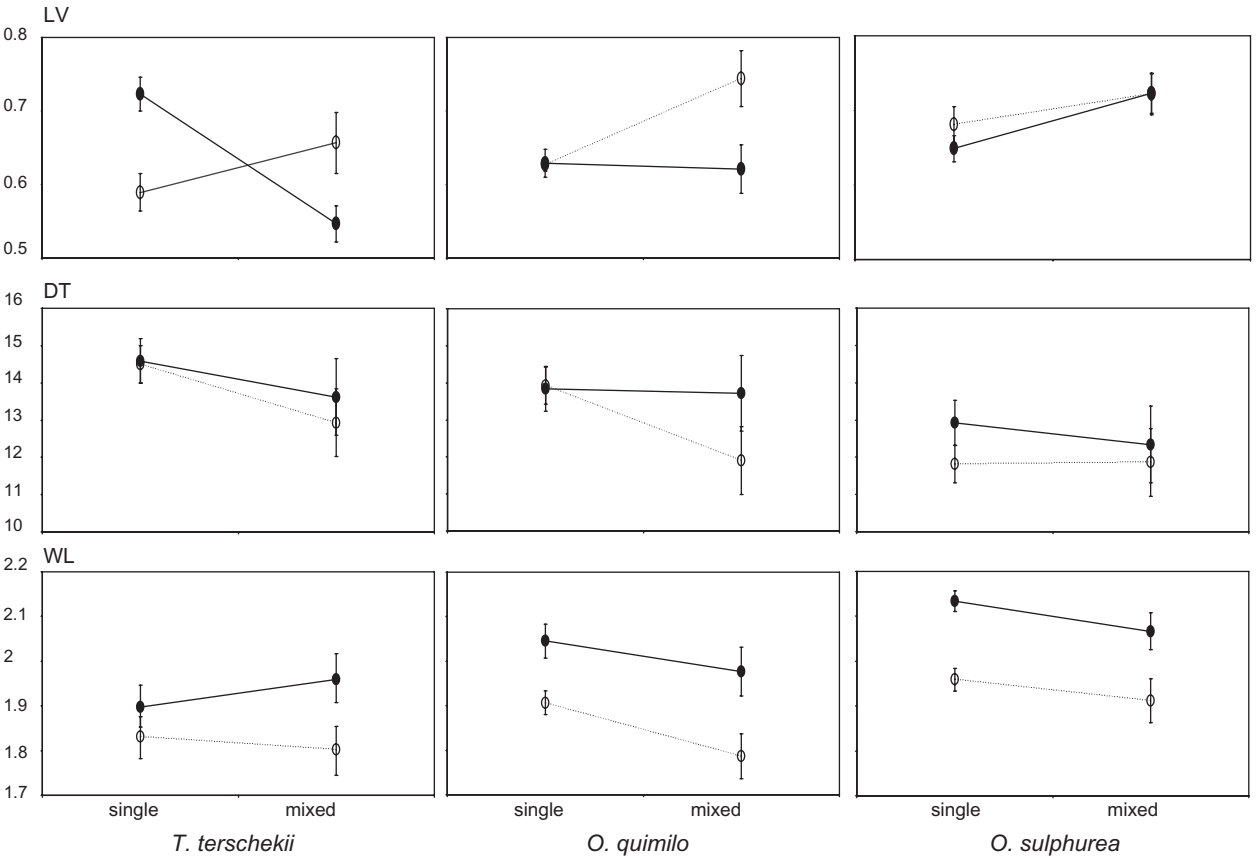

Fig. 2. Mean and standard deviation of larval viability in percentage of survival (LV), developmental time in days (DT) and wing length in millimetre (WL) of *Drosophila buzzatii* (open circle) and *D. koepferae* (filled circle) reared in single and mixed species culture reared in *Trichocereus terschekii* (left column), *Opuntia quimilo* (central column) and *O. sulphurea* (right column).

(Fanara *et al.* 1999, 2004). For example, concentration of carbohydrates in prickly pears has been shown to be higher than in columnar cacti inhabiting the desert of Sonora (Kircher 1982; Fogleman and Abril 1990). In addition, differences in the relative abundance and diversity of the yeasts associated with the decaying process of cacti (Starmer *et al.* 1990) are also important factors that may differentiate *T. terschekii* and *Opuntia* as breeding sites. In addition, the presence of toxic compounds produced by the host or different waste products produced by a competing species, or a combination of both, may also affect the suitability of the host plant (Cockburn 1991; Santos *et al.* 1992a; Joshi *et al.* 1996; James & Partridge 1998).

Interspecific competition is a major feature in the ecology of insect communities exploiting ephemeral and fragmented resources (Inouye 1999; Krijger *et al.* 2001). In this sense, our results suggest that interspecific competition offers only a partial explanation for the different records of emergence of *D. koepferae* and *D. buzzatii* from naturally occurring rotting cacti (Hasson *et al.* 1992; Fanara *et al.* 1999). For most traits analysed differences between flies reared in single and mixed species cultures were highly significant, suggesting the effect of the presence of heterospecifics. Flies may avoid the negative effect that the presence of heterospecific competitors may cause by shortening the time needed to reach the pupal stage (Bakker 1961; Prasad *et al.* 2001), along with a concomitant reduction in body size. All in all, these results point out that interference (for instance due to the presence of waste metabolites) as well as exploitative competition would play an important role during interspecific competition. However, it is interesting to note that the effect of interspecific competition varied not only across species but also depended on factors related to rearing conditions (cactus and density) and the trait analysed. On the one hand, it may be argued that the decrease in DT in combination with the reduction observed in wing size, in both *Opuntia* hosts, might be an indication of the susceptibility of these cactophilic species to the presence of conspecifics but not affecting survival. On the other hand, the effect of the type of culture involved different patterns of variation in *D. buzzatii* and *D. koepferae*, particularly in

doi:10.1111/j.1442-9993.2008.01833.x

columnar cacti. In the former, this factor was only significant for DT, suggesting a non-plastic response to the presence/absence of heterospecific competitors for LV and WL, whereas in *D. koepferae* flies attained a larger size, faster DT and higher survival when *D. buzzatii* larvae were present in the same vials. These results suggest that there is an interspecific difference in the efficiency of converting food into biomass that depends on the cactus host.

The results of the present study are in agreement with those obtained in similar experiments in *D. buzzatii* and the distantly related *D. aldrichi* (Krebs & Barker 1991, 1993; Krebs *et al*. 1992). *Drosophila buzzatii* outperformed *D. aldrichi* when both species competed for the same resources, while the latter had a better performance than *D. buzzatii* when competition was restricted to conspecifics. However, it is important to point out that in these studies *D. buzzatii* flies were derived from Australian natural populations and were reared in different cactus media than those used in the experiments reported herein. Nevertheless, we cannot assert that *D. buzzatii* per se is a better interspecific competitor than other cactophilic species, although the evidence appears convincing.

Finally, we would like to address a question related to the differential evolutionary success of *D. buzzatii* and *D. koepferae* as judged by their present geographical distribution. In this paper we have shown that *D. koepferae* has the ability to utilize *O. quimilo* as a natural breeding site (see also Hasson *et al*. 1992). Ecological surveys have shown that *D. buzzatii* is the only species of the buzzatii complex that emerges primarily from *Opuntia* rotting cladodes, while the other members of the cluster: *D. koepferae*, *D. serido*, *D. borborema, D. antonietae, D. gouviei* and *D. seriema* emerge predominantly from columnars (Hasson *et al*. 1992; Manfrin & Sene 2006) and use prickly pears only as secondary breeding sites (Hasson *et al*. 1992; Fanara *et al*. 1999). Thus, emergence records suggest that *O. quimilo* can be considered a novel resource for *D. koepferae* as this cactus is abundant in the Chaco phytogeographical province where *D. buzzatii* predominates and *D. koepferae* is rare. The ability to switch to a novel resource may be critical in the colonization of new habitats (Spicer & Jaenike 1995; Fry 2003; Parsons & Robinson 2006), wherein there are two phases: host plant selection and exploitation of the host during the expansion of a species. During host plant selection females use volatile compounds as cues to locate a suitable breeding site whereas the utilization of the host plant refers to the ability of the larvae to make use of it (Fogleman & Abril 1990). Our results suggest that neither the performance of *D. koepferae* nor the effect of competition can explain the absence of *D. koepferae* in areas where *O. quimilo* is the dominant cactus species in Chaco phytogeographical province. Considering all the available evidence it seems reasonable to propose that the greater evolutionary success of *D. buzzatii* in the arid zones of southern South America (Fontdevila 1989; Hasson *et al*. 1992; Piccinali *et al*. 2004) cannot be attributed to differential competitive ability. Then, other features should be considered to explain the differences between *D. buzzatii* and *D. koepferae* in the distribution and colonization of novel hosts. In this sense, it has been shown that *D. buzzatii* and *D. koepferae* differ in their willingness to accept different substrates as oviposition sites (Fanara & Hasson 2001). Thus, future work in *D. buzzatii* and *D. koepferae* should address a simple question: is there a correlation between host preference and performance in specific hosts?

ACKNOWLEDGEMENTS

We wish to thank to two anonymous reviewers and M. Bull for comments that helped to improve this paper. We also thank other members of our research group for helpful discussion. This work was supported by Universidad de Buenos Aires, CONICET and ANPCyT grants. VW and LO are recipients of scholarships awarded by CONICET. JJF and EH are fellows of CONICET (Argentina).

REFERENCES

Bakker K. (1961) An analysis of factors which determine success in competition for food among larvae of *Drosophila* melanogaster. *Arch. Neerl. Zool.* **14,** 200–81.

Baldal E. A., van der Linde K., van Alphen J. J., Brakefield P. M. & Zwaan B. J. (2005) The effects of larval density on adult life-history traits in three species of *Drosophila*. *Mech. Ageing Dev.* **126,** 407–16.

Barker J. S. F. & Starmer W. T., eds. (1982) *Ecological Genetics and Evolution. The Cactus-Yeast-Drosophila Model System*. Academic Press, Sydney.

Cockburn A. (1991) *An Introduction to Evolutionary Ecology*. Blackwell Scientific Publications, Oxford.

David J. (1962) A new medium for rearing *Drosophila* in axenic conditions. *DIS* **36,** 128.

Diehl S. R. & Bush G. L. (1989) The role of the habitat preferences in adaptation and speciation. In: *Speciation and Its Consequences* (eds D. Otte & J. A. Endler), pp. 345–65. Sinauer, Sunderland.

Etges W. J. & Heed W. B. (1987) Sensitivity to larval density in populations of *Drosophila mojavensis*: influences of host plant variation on components of fitness. *Oecologia* **71,** 375–81.

Fanara J. J. & Hasson E. (2001) Oviposotopn acceptance and fecundity schedule in the cactophilic sibling species *Drosophila buzzatii* and *D. koepferae* on their natural host. *Evolution* **55,** 2615–19.

Fanara J. J., Hasson E., Rodríguez C., Santos M. & Fontdevila A. (1996) The evolutionary history of *Drosophila buzzatii*. XXXIII. Are *Opuntia* hosts a selective factor for the inversion polymorphism? *Heredity* **77,** 500–8.

doi:10.1111/j.1442-9993.2008.01833.x

Fanara J. J., Hasson E. & Fontdevila A. (1999) Oviposition preference, viability, developmental time and body size in the cactophilic sibling species *Drosophila koepferae* and *D. buzzatii* in association to their natural hosts. *Evol. Ecol.* **13,** 173–90.

Fanara J. J., Mensch J., Folguera G. & Hasson E. (2004) Developmental time and thorax length differences between the cactophilic species *Drosophila buzzatii* and *D. koepferae* reared in different natural hosts. *Evol. Ecol.* **18,** 203–14.

Fogleman J. C. & Abril J. R. (1990) Ecological and evolutionary importance of host plant chemistry. In: *Ecological and Evolutionary Genetics of Drosophila* (eds J. F. S. Barker, W. T. Starmer & R. J. MacIntyre), pp. 121–43. Plenum Press, New York.

Fontdevila A. (1989) Founder effects in colonizing populations the case of *Drosophila buzzatii*. In: *Evolutionary Biology of Transient Unestable Populations* (ed. A. Fontdevila), pp. 74–95. Springer-Verlag, Heidelberg.

Fontdevila A., Pla C., Hasson E. *et al.* (1988) *Drosophila koepferae*: a new member of the *Drosophila serido* (diptera-Drosophilidae) superspecies taxon. *Ann. Entomol. Soc. Am.* **81,** 380–5.

Fry J. D. (2003) Detecting ecological trade-offs using selection experiments. *Ecology* **84,** 1672–8.

Hasson E., Naveira H. & Fontdevila A. (1992) The breeding sites of the Argentinean species of the *Drosophila mulleri* complex (subgenus *Drosophila*-repleta group). *Rev. Chil. Hist. Nat.* **65,** 319–26.

Hopf F. A., Valone T. J. & Brown J. H. (1993) Competition theory and the structure of ecological communities. *Evol. Ecol.* **7,** 142–54.

Inouye B. D. (1999) Estimating competition coefficinets: strong competition among three species of frugivorous flies. *Oecologia* **120,** 588–94.

James A. C. & Partridge L. (1998) Geographic variation in competitive ability in *Drosophila melanogaster*. *Am. Nat.* **151,** 530–7.

Joshi A., Knight C. D. & Muller L. D. (1996) Genetics of larval urea tolerance in *Drosophila melanogaster*. *Heredity* **77,** 33–9.

Kircher H. W. (1982) Chemical composition of cacti and its relationship of Sonora Desert. In: *Ecological Genetics and Evolution* (eds J. S. F. Barker & W. T. Starmer), pp. 143–58. Academic Press, Sydney.

Krebs R. A. & Barker J. S. F. (1991) Coexistence of ecologically similar colonising species. Intra- and interspecific competition in *Drosophila aldrichi and D. buzzatii*. *Aust. J. Zool.* **39,** 579–93.

Krebs R. A. & Barker J. S. F. (1993) Coexistence of ecologically similar colonising species. II. Populations differentiation in *Drosophila aldrichi* and *D. buzzatii* for competitive effects and responces at different temperatures and allozyme variation in *D. aldrichi*. *J. Evol. Biol.* **6,** 281–98.

Krebs R. A., Barker J. S. F. & Armstrong T. P. (1992) Coexistence of ecologically similar colonising species III. *Drosophila aldrichi* and *D. buzzatii*: larval perfomance on, and adult preference for, three *Opuntia* cactus species. *Oecologia* **92,** 362–72.

Krijger C. L., Peters Y. C. & Sevenster J. C. (2001) Competitive ability of neotropical *Drosophila* predicted from larval development times. *Oikos* **92,** 325–32.

Manfrin M. H. & Sene F. M. (2006) Cactophilic *Drosophila* in South America: a model for evolutionary studies. *Genetica* **126,** 57–75.

Margulis L. & Fester R., eds (1991) *Symbiosis as a Source of Evolutionary Innovation: Speciation and Morphogenesis*. MIT Press, Cambridge

Maynard Smith J. & Szathmary D. E. (1995) *The Major Transitions in Evolution*. Freeman, San Francisco.

Morris D. W. (1999) Has the ghost of competition passed? *Evol Ecol. Res.* **1,** 3–20.

Nunney L. (1990) *Drosophila* on oranges: colonization, competition, and coexistance. *Ecology* **71,** 1904–15.

Parsons K. J. & Robinson B. W. (2006) Replicated evolution of integrated plastic responses during early adaptive divergence. *Evolution* **60,** 801–13.

Piccinali R., Aguadé M. & Hasson E. (2004) Comparative molecular population genetics of the locus xdh in the cactophilic sibling species *Drosophila buzzatii* and *D. koepferae* in the arid zones of Argentina. *MBE* **21,** 117–28.

Powell P. (1997) *Progress and prospects in evolutionary biology. The Drosophila Model*. Oxford University Press, New York.

Prasad N. G., Shakarad M., Anitha D., Rajamani M. & Joshi A. (2001) Correlated responses to selection for faster development and early reproduction in *Drosophila*: the evolution of larval traits. *Evolution* **55,** 1363–72.

Quezada Diaz J. E., Laayouni H., Leibowitz A., Santos M. & Fontdevila A. (1997) Breeding structure of *Drosophila buzzatii* in relation to competition in prickly pears (*Opuntia ficus-indica*). *Gen. Sel. Evol.* **29,** 367–82.

Rainey P. B. & Travisano M. (1998) Adaptive radiation in a heterogeneous environment. *Nature* **394,** 69–72.

Rausher M. D. (1984) The evolution of habitat selection in subdivided populations. *Evolution* **38,** 596–608.

Robertson F. W. (1987) Variation of body size within and between wild populations of *Drosophila buzzatii*. *Genetica* **72,** 111–25.

Rodriguez C., Fanara J. J. & Hasson E. (1999) Inversion polymorphism, longevity, and body size in a natural population of *Drosophila buzzatii*. *Evolution* **53,** 612–20.

Rohlfs M. & Hoffmeister T. S. (2004) Spatial aggregation across ephemeral resource patches in insect communities: an adaptive response to natural enemies? *Oecologia* **140,** 654–61.

Roper C., Pignatelli P. & Partridge L. (1996) Evolutionary responses of *Drosophila melanogaster* life history to differences in larval density. *J. Evol. Biol.* **9,** 609–22.

Rosenzweig M. L. (1991) Habitat selection and population interactions: the search for mechanism. *Am. Nat. (Suppl.)* **137,** 5–28.

Rosewell B., Shorrocks B. & Edwards K. (1990) Competition on a divided and ephemeral resource: testing the assumptions. I. Aggregations. *J. Anim. Ecol.* **59,** 977–1001.

Ruiz A. & Wasseman M. (1993) Evolutionary cytogenetics of the *Drosophila buzatii* species complex. *Heredity* **70,** 582–96.

Sanders A. E., Scarborough C., Layen S. J., Kraaijeveld A. R. & Godfray H. C. (2005) Evolutionary change in parasitoid resistence under crowded conditions in *Drosophila melanogaster*. *Evolution* **59,** 1292–9.

Santos M., Fowler K. & Partridge L. (1992a) On the use of tester stocks to predict the competitive ability of genotypes. *Heredity* **69,** 489–95.

Santos M., Ruiz A., Quezada Diaz J. E., Barbadilla A. & Fontdevila A. (1992b) The evolutionary history of *Drosophila buzzatii*. 20. Positive phenotypic covariance between field adult fitness and body size. *J. Evol. Biol.* **5,** 403–22.

Santos M., Borash D., Joshi A., Bounlutay N. & Muller L. (1997) Density-dependent natural selection in *Drosophila*:

doi:10.1111/j.1442-9993.2008.01833.x

evolution of growth rate and body size. *Evolution* **51,** 420–32.

Shiotsugu J., Leroi A. M., Yashiro H., Rose M. R. & Mueller L. D. (1997) The symmetry of correlated selection responses in adaptive evolution: an experimental study using *Drosophila*. *Evolution* **51,** 163–72.

Shorrocks B. & Rosewell J. (1987) Spatial patchiness and community structure: coexistence and guild size of *Drosophila* on ephemeral resources. In: *The Organization of Communities: Past and Present* (eds J. H. R. Gee & P. S. Giller), pp. 29–51. Blackwell Scientific Publications, Oxford.

Sokal R. R. & Rohlf F. J. (1985) *Biometry*. W.H. Freeman, New York.

Spicer G. S. & Jaenike J. (1995) Phylogenetic analysis of breeding site use and alpha-amanatin tolerance within the *Drosophila quinaria* species group. *Evolution* **50,** 2328–77.

Starmer W. T., Lachance M., Phaff H. J. & Heed W. B. (1990) The biogeography of yeast associated with decaying cactus tissue in North America, the Caribean, and Northerb Venezuela. *Evol. Biol.* **24,** 115–90.

StatSoft Inc (2001) *Statistica User's Guide*. Release 6.0 Edition. Statsoft Inc, Tulsa.

Thomas R. H. (1993) Ecology of body size in *Drosophila buzzatii*: untangling the effects of temperature and nutrition. *Ecol. Entomol.* **18,** 84–90.

Thompson J. N. (1999) The evolution of species interactions. *Science* **284,** 2116–18.

Thompson J. N. & Cunningham B. M. (2002) Geographic structure and dynamics of coevolutionary selection. *Nature* **417,** 735–8.

Vilela C. A. (1983) A revision of the *Drosophila* species group. (Diptera-Drosophilidae). *Rev. Bras. Entomol.* **27,** 1–114.

Wasserman M. (1992) Cytological evolution of the *Drosophila repleta* species group. In: *Drosophila Inversion Polymorphism* (eds C. B. Krimbas & J. R. Powell), pp. 455–550. CRC Press, Boca Ratón.

doi:10.1111/j.1442-9993.2008.01833.x