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Oviposition preference and life history traits in cactophilic *Drosophila koepferae* and *D. buzzatii* in association with their natural hosts

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Abstract. *Drosophila koepferae* and *D. buzzatii* are two closely related cactophilic species inhabiting the arid lands of southern South America. Previous studies have shown that *D. buzzatii* breeds primarily on the necrotic cladodes of several *Opuntia* cacti and *D. koepferae* on the rotting stems of columnar cacti of the genera *Trichocereus* and *Cereus*. In this paper, we analyze the patterns of host plant utilization in a locality where both *Drosophila* species are sympatric. Field studies showed an absence of differential attraction of adult flies to the rots of two major host cacti: *O. sulphurea* and *T. terschekii*. However, the proportion of *D. buzzatii* flies emerged from the rotting cladodes of *O. sulphurea* was significantly higher than in *T. terschekii*. In laboratory experiments, egg to adult viability in single species cultures varied when both *Drosophila* species were reared in media prepared with *O. sulphurea* or *T. terschekii*. In addition, between-species comparisons of flies emerged from single species cultures showed that *D. buzzatii* adults were smaller and developed faster than *D. koepferae*. Furthermore, analysis of flies emerged in mixed species cultures showed differences in oviposition preference and oviposition behavior. We discuss the observed between-species differences and suggest that these traits are the result of adaptation to specific patterns of spatial and temporal predictability of their respective preferred host plants: columnar are less dense and less ephemeral resources, whereas the opuntias are more abundant, and fast rotting cacti.

Key words: body size, cactophilic Drosophila, developmental time, host plants, oviposition preference

Introduction

Host plant specialization seems to be causally related to the astonishing diversity of phytophagous insects (Jaenike, 1990). In order to understand the origin and maintenance of diversity in such groups, it may be necessary to understand the underlying causes of host plant utilization. Evolutionary changes associated with host shifts may be adaptive, i.e. driven by natural selection, therefore, life history traits lay at the base of our understanding of the processes related to the evolution of host plant utilization, along with the knowledge of chemical, physical and distributional properties of the hosts plants. Thus, adaptation to new hosts may lead to life history evolution (Mitler and Futuyma, 1983) and, to divergent adaptive strategies in species interacting in heterogeneous environments.

The study of adaptive traits is complex because they are the result of three different and interacting levels: the phenotype, the genotype and the environment (Templeton *et al.*, 1990; Wilson, 1997; Goodnight and Stevens, 1997). The cactus-yeast-*Drosophila* model system has proved to be an excellent subject for evolutionary studies in this regard (Barker and Starmer, 1982; Heed and Mangan, 1986). The knowledge of the ecology of several cactophilic species allows highly specific sampling procedures (Carson and Heed, 1983; Heed and Mangan, 1986) for the study of life history trait variation under diverse ecological conditions (Prout and Barker, 1989; Etges, 1990, 1993; Krebs and Barker, 1991, 1993; Krebs *et al.*, 1992; Santos *et al.*, 1992; Hasson *et al.*, 1993; Norry *et al.*, 1995).

Drosophila buzzatii and *D. koepferae* are two sibling cactophilic species belonging to the buzzatii complex (repleta group) (Wasserman, 1992; Ruiz and Wasserman, 1993), that breed and feed on the necroses of several cactus species (Fontdevila *et al.*, 1988; Hasson *et al.*, 1992; Fanara, 1995). Their species' range overlap in the arid regions of southern South America (Fontdevila *et al.*, 1988; Hasson *et al.*, 1992; Fanara, 1995). Although, *D. buzzatii* and *D. koepferae* exhibit a certain degree of niche overlap (Hasson *et al.*, 1992), the former is largely associated with prickly pears (genus *Opuntia* subfamily *Opuntioideae*), while *D. koepferae* breeds primarily on columnar cacti of the genera *Cereus* and *Trichocereus* (subfamily *Cereoideae*; see Kiesling, 1975 for a description of Argentinian cacti). The study of host plant utilization in *D. buzzatii* and *D. koepferae* has an added significance because we can address the extent of life history evolution resulting from adaptation to alternative hosts after their recent split from a common ancestor.

Environmental heterogeneity is a necessary condition for habitat selection and, in this sense Opuntia and columnar cacti may represent different niches for *Drosophila* due to differences in chemical composition (Kircher, 1982; Fogleman and Abril, 1990), and in the microflora (yeasts and bacteria) associated with the decaying process of cactus tissues (reviewed in Starmer *et al.*, 1990). In addition, the interaction between cactus and yeasts may also lead to quite different profiles of volatile compounds which *Drosophila* adults use for locating suitable breeding sites (Parsons, 1983; Fogleman and Abril, 1990; Armstrong, 1992). Thus, opuntias and columnars may be considered as different compartments of a heterogeneous environment setting up the stage for habitat selection.

Some authors have suggested that females may choose oviposition sites in which their progeny's performance is maximized (Rausher, 1983; Singer *et al.*, 1988), whereas others claim that preference and performance may not always be correlated (Jaenike, 1989, 1990; Courtney and Kibota, 1990; Fox, 1993). Since performance may, in turn, determine the evolution of host plant utilization and consequently niche width (Diehl and Bush, 1989; Etges, 1993; Joshi and Thompson, 1995, 1997; Hawthorne, 1997), the expansion of a species to a new niche will depend on the correlations between performances in different hosts. Negative correlations may impose a genetic constraint to niche expansion (Rausher, 1984, 1988; Diehl and Bush, 1989), and non significant correlations among hosts may not be a sufficient cause for the expansion to new niches since it could be limited by ecological, behavioral and/or phylogenetic constraints (Hoffman, 1985; Jaenike, 1990; Lederhause *et al.*, 1992; Futuyma *et al.*, 1993, 1995).

The objective of the present paper is to characterize the patterns of host plant utilization of *D. buzzatii* and *D. koepferae* by means of the study of differential attraction to and emergence from two different host plants in the field. In addition, we analyze in culture media prepared with rotting materials of *O. sulphurea* or *T. terschekii*, egg to adult viability, developmental time and thorax length, traits that are known to be influenced by the environmental conditions faced by the developing larvae (Etges and Heed, 1987; Ruiz and Heed, 1988; Cavicchi *et al.*, 1989; McCabe and Partridge, 1997), and explore the possible role of oviposition preference.

Materials and methods

On the basis of cytological evidence, Ruiz and Wasserman (1993) erected the buzzatii complex in which *D. buzzatii* and *D. koepferae* were included. Females of both species are morphologically indistinguishable, while males can be readily told apart by examining the morphology of their aedeagus (Vilela, 1983). Different chromosomal inversions became fixed in *D. buzzatii* (5g) and in *D. koepferae* ($2j^9$) after divergence from their hypothetical common ancestor (Wasserman, 1992), thus polytene chromosomes can be also used to differentiate species.

The locality studied

Most of the flies analyzed in the present study were collected in the locality named Quilmes (Argentina) in previous studies (Hasson *et al.*, 1992, 1995) located in the very arid Monte Phytogeographic Province. The site of collection is 2000 m above sea level, 20 km West of the town Amaicha del Valle

(Tucumán Province) and 200 km west of San Miguel de Tucumán. *Trichocereus terschekii* (cardón) and *O. sulphurea*, are the only cactus species present. Additional collections were performed in the town of Amaicha del Valle where *O. ficus-indica* and *O. sulphurea* are potential hosts for cactophilic *Drosophila.*

Field studies: attraction to host plants and emergence records

We studied differential utilization of *T. terschekii* and *O. sulphurea* rots by analyzing samples of flies collected with traps prepared with rotting tissues of both cactus species and the commonly used fermented banana baits (Carson and Heed, 1983). Adult flies were aspirated directly from the baits or caught by net sweeping.

Two collections of adult flies were made in Quilmes and Amaicha del Valle, the first immediately after the rainy season in May 1992 and the second before the subsequent rainy season in December 1993. In Quilmes, all collections were carried out using the three types of bait mentioned above, while in Amaicha del Valle only banana baits were used. Two daily collections of adult flies were conducted, one during the morning from 8 to 10 am and the other at sunset from 5 to 7 pm). All collected flies were transported to the laboratory, sorted by sex and classified to species.

Rotting stems of *T. terschekii* and rotting cladodes of *O. sulphurea* were collected, wrapped in paper in the field and brought to the laboratory where they were placed in 1.5 l plastic containers. All flies emerging during the following two weeks were aspirated every other day from the bottles and classified to species whenever possible (see below). All flies emerged in the same container were kept together for 8 days in groups of 40 individuals in bottles with laboratory culture medium (see below). This procedure ensured that flies of both species reached sexual maturity and all females were inseminated. Subsequently, flies were sorted by sex and females used to established isofemale lines.

Bait collected and emerged males were classified to species by inspection of their genitalia, while females were identified by the analysis of the polytene chromosomes of one progeny larva from each isofemale line.

Laboratory experiments

Single species cultures

Seventy five *D. koepferae* and 50 *D. buzzatii* isofemale lines were maintained in bottles with 30 ml of laboratory medium for one generation. Two virgin males and females of each line were used to obtain outbred stocks of each species. Thus, the individuals employed for the experiments described below corresponded to the second laboratory generation of field caught females.

For each species, 200 pairs of sexually mature flies were placed in egg-collecting chambers. Egg laying medium (agar 2% + 5 ml of 3 parts of ethanol and 1 part of 60% acetic acid) was poured into medium size petri dishes. Dehydrated baker's yeast was used to stimulate oviposition. Large number of eggs were collected on the agar surface and then sterilized with 50% clorox for 3 min (Starmer and Gilbert, 1982). For each species, two egg-collecting chambers were set up.

Three batches of 100 eggs were placed on sterile agar and allowed to hatch in order to obtain estimates of egg-hatchability in *D. buzzatii* and *D. koepferae*, by scoring the number of first instar larvae.

The remaining eggs were placed on Petri dishes with sterile agar and allowed to hatch. Batches of 40 first instar larvae were seeded in culture vials containing 6 ml (optimal density according to Fanara *et al.*, 1995) of two different types of medium: (a) David's (1962) killed yeast laboratory medium and (b) two 'natural media' prepared with fresh cactus tissues of *T. terschekii* or *O. sulphurea*. Pieces of cactus were ground into a blender and 6 g poured into each glass vial. Media were then sterilized in an autoclave and after cooling each vial was inoculated with 0.1 ml of fermenting juice obtained from naturally occurring rots. The fermenting juice of each cactus species was obtained from several natural rots and maintained for further experiments by adding 10 g of fresh cactus every two weeks. First instar larvae were seeded 24 h after the inoculation of the microorganisms.

For each *Drosophila* species, cactus, and laboratory medium, five replicated vials were run. Two *D. buzzatii* vials in *T. terschekii* medium were lost due to mold contamination. In each vial, viability was estimated as the proportion of emerging adults relative to the number of first instar larvae. Developmental time (DT) was measured as the time elapsed since the transfer of first instar larvae to the vials until adult emergence. Finally, thorax length (TL) was measured to the nearest 0.031 mm from the anterior margin to the tip of the scutellum in lateral view.

Mixed cultures

The same outbred stocks described in the preceding section were employed in a set of experiments designed to explore the occurrence of preferential oviposition in *D. buzzatii* and *D. koepferae*.

For each *Drosophila* species two mating chambers were set up with 450 flies (150 virgin females and 300 males) for two hours. According to Norry *et al.* (1995), two hours is sufficient time for all *D. buzzatii* females to be inseminated and preliminary observations suggest the same for *D. koepferae* (Fanara, 1995). Subsequently, flies were recovered from the chambers, males discarded, and females employed for the next phase of the experiment.

Groups of 150 inseminated females were introduced into egg collecting chambers in a proportion of 7 *D. koepferae*: 3 *D. buzzatii*, identical to the proportions of flies attracted to *O. sulphurea* and *T. terschekii* field traps (see Results).

Four egg-collecting chambers were set up, in two of them the juice of rotting *O. sulphurea* was sprinkled onto the agar surface to stimulate oviposition, while in the other two the juice of rotting *T. terschekii* was used. Petri dishes containing egg-collecting medium were replaced daily and eggs collected during three consecutive days.

Eggs were allowed to hatch and batches of 40 first-instar larvae were seeded in vials containing laboratory or one of the previously described 'natural media'. In each case first-instar larvae were seeded in the medium prepared with the same cactus employed for oviposition.

Thirteen vials were obtained from *O. sulphurea* chambers and 11 vials from *T. terschekii* chambers during the first day of egg collection. Five and 9 vials were seeded with the larvae collected during the second day from *O. sulphurea* and *T. terschekii* chambers, respectively. Finally, 4 and 7 vials were obtained from *O. sulphurea* and *T. terschekii* chambers respectively, on the third day.

All experiments were conducted at 25 °C.

Results

Field collections

More than three thousand adult flies were collected using banana baits and rots of *O. sulphurea* and *T. terschekii* in Quilmes (Table 1). Two-thirds of the flies were caught with banana baits, while similar number of flies were attracted to the cactus baits. However the specificities of each kind of trap were very

Table 1. Number of *Drosophila* adult flies collected with banana, *Opuntia sulphurea* and *Trichocereus terschekii* baits and emerged from rotting pockets of both cactus species in Quilmes (May 1992)

Species	Collected			Emerged	
	Banana	*O. s.*	*T. t.*	*O. s.*	*T. t.*
D. buzzatii males	34	37	37	64	43
D. koepferae males	400	78	80	33	612
Percent of *D. buzzatii* males	7.83	32.17	31.62	65.98	6.56
D. buzzatii + *D. koepferae* females	357	95	123	142	750
D. serenensis	822	74	238	1	442
D. melanogaster	70	54	23	8	0
Drosophila sp.	287	182	88	0	0
Total	1970	520	589	248	1847

different (Table 1). Besides *D. koepferae* and *D. buzzatii*, the cactophilic *D. serenensis*, *D. melanogaster* and some non-identified species were also attracted to the baits. Non-cactophilic species constituted only 18% of the flies caught with banana and *T. terschekii*, while they surprisingly made up 45% of the collections with *O. sulphurea*. About 74% of the cactophilic flies collected on *O. sulphurea* belonged to the pair *D. koeferae–D. buzzatii*, while they made up 50% of the collections on banana and *T. terschekii*. Eight percent of the males, belonging to the sibling pair, collected in banana baits were *D. buzzatii*, while this proportion was 32% in *O. sulphurea* and *T. terschekii*. When we consider only *D. buzzatii* and *D. koepferae*, the number of males of each species trapped with *O. sulphurea* were not significantly different from the proportions attracted to *T. terschekii* ($\chi^2 = 0.007$, $d.f. = 1$, $P = 0.97$). However, the differences between natural traps and banana were highly significant ($\chi^2 = 64.5$, $d.f. = 1$, $p < 0.0001$).

We employed 15 buckets containing fermented bananas, 72 groups of *O. sulphurea* rots and 22 traps made with rotting *T. terschekii* over 7 days. The average number of males of each species trapped per collecting unit of banana, *O. sulphurea* and *T. terschekii*, were for *D. buzzatii* 2.3, 0.5 and 1.7 respectively, and for *D. koepferae* 26.6, 1.1 and 3.6, respectively. These data illustrate that the effectiveness of banana baits was greater than natural traps especially for *D. koepferae*, and that rots of *T. terschekii* are more effective than *O. sulphurea*.

Simultaneous collections on banana baits were obtained in the neighbouring locality of Amaicha del Valle (337 flies), where only *Opuntia* hosts (*O. sulphurea* and *O. ficus-indica*) are present. *D. buzzatii* represented 80% of the flies caught, 4% were *D. serenensis*, 8% *D. melanogaster* and 8% non-identified *Drosophila* species. *D. koepferae* was absent in this collections.

In December 1993, we tried to repeat the same collecting scheme, however this was not only possible since *O. sulphurea* rots were not available at the time of these collections. Out of a total of 478 flies collected, 418 (87%) belonged to *D. buzzatii* (1%) or *D. koepferae* (99%), while *D. serenensis* represented only 1%. A consistently higher proportion of *D. melanogaster* was caught with banana baits in this collection.

Rot collections and rearing records

More than two hundred fifty flies emerged from twenty rotting cladodes of *O. sulphurea* rots and 2000 adult flies from 7 rotting stems of *T. terschekii* collected in Quilmes in May 1992 (Table 1). The emergence window, i.e. the time elapsed from the emergence of the first until the last fly, varied from 6 to 12 days for *Opuntia* and from 2 to 14 days for cardón.

More than 96% of the flies emerged from *O. sulphurea* belonged to the pair of sibling species (Table 1). Among them 34% of the emerged flies were

D. koepferae. Besides *D. buzzatii* and *D. koepferae*, small numbers of *D. serenensis* and *D. melanogaster* emerged from *O. sulphurea*.

The identification of the flies reared from cardón showed a very different picture. Seventy six percent of the emerged flies belonged to the pair *D. buzzatii–D. koepferae* and 24% to *D. serenensis*. *D. koepferae* was the most abundant accounting for 93% of the flies belonging to the pair of sibling species (Table 1).

The emergence records of *D. buzzatii* and *D. koepferae* from *O. sulphurea* and *T. terschekii* were significantly different ($\chi^2 = 244.42$, $d.f. = 1, p < 0.0001$), confirming that *D. buzzatii* emerged preferentially from *O. sulphurea* and *D. koepferae* from cardón.

In summary, field results showed that the proportions of *D. buzzatii* and *D. koepferae* attracted to *O. sulphurea* and *T. terschekii* were similar and emergence records significantly different. Moreover, 67.8% of the flies collected on *O. sulphurea* were *D. koepferae*, while this species represented only 34% of the flies emerged from *O. sulphurea* rots, a difference that proved to be significant ($\chi^2 = 24.1$, $d.f. = 1$, $p < 0.0001$). Similarly, in cardón the proportion of *D. koepferae* differed significantly ($\chi^2 = 67.2$, $d.f. = 1$, $p < 0.0001$) between attracted (68%) and emerged (93%) males.

Laboratory experiments

Single species experiments

Egg to adult viability. Mean egg hatchability was high in *D. buzzatii* (81% ± 1) and *D. koepferae* (80% ± 2) and not significantly different between species ($t = 1.2$, $p = 0.28$).

D. koepferae showed an unexpectedly low viability in laboratory medium (only 26.1% of the larvae reached adulthood, SD = 0.08) and higher viabilities in *T. terschekii* (76.0 ± 0.06) and *O. sulphurea* (67.7 ± 0.08). On the other hand, *D. buzzatii* was more viable in laboratory medium (62.5 ± 0.03) and exhibited lower viabilities in cardón (47.8 ± 0.10) and *O. sulphurea* (58.2 ± 0.10). The viability of *D. buzzatii* in *Opuntia* medium was slightly higher than the values reported in Krebs *et al.* (1992) in Australian *D. buzzatii* flies reared under similar conditions.

Proportions of males and females did not depart from the expected 1:1 sex ratio in any case ($\chi^2 = 1.6 \times 10^{-4}$ and $\chi^2 = 1.5 \times 10^{-3}$ for *D. buzzatii* in *O. sulphurea* and *T. terschekii* respectively, and $\chi^2 = 4.3 \times 10^{-3}$ and $\chi^2 = 3.2 \times 10^{-3}$ for *D. koepferae* in *O. sulphurea* and *T. terschekii* respectively; $d.f. = 1$ in all tests).

Viability data in laboratory medium were excluded from the ANOVA designed to investigate the effect of cactus hosts (fixed factor), *Drosophila* species (fixed) and chambers (random) nested in cactus hosts, on viability. Prior to the ANOVA viability data were angularly transformed using the expression arcsin $(p)^{1/2}$ (where p is the percent of survivors). Significant differences were detected between *Drosophila* species ($F_{1,2} = 353.4$, $p < 0.003$) while neither the effect of cactus hosts ($F_{1,2} = 0.04$, $P = 0.85$) nor chambers within cactus ($F_{2,30} = 1.04$, $p = 0.37$) were significant. Moreover, the *Drosophila* × cactus interaction was highly significant ($F_{1,2} = 103.1$, $p = 0.009$) suggesting that viability is not independent of cactus media.

In order to determine the causes of the significant interaction observed, viability data of each species were analyzed by means of one way ANOVAs with cactus hosts as fixed effect for each *Drosophila* species separately. *D. koepferae* was significantly more viable in cardón than in *Opuntia* ($F_{1,16} = 9.1$, $p = 0.008$), whereas *D. buzzatii* reared in *O. sulphurea* were only marginally more viable than in cardón ($F_{1,14} = 4.5$, $p = 0.053$).

Thorax length. Mean thorax lengths of *D. buzzatii* and *D. koepferae* reared in both cactus media are presented in Table 2. Thorax length data were analyzed by means of a multifactorial ANOVA, with *Drosophila* species and cactus hosts as fixed factors and replicates (random) nested in chambers (random) and chambers nested in cactus hosts. Independent tests were performed for males and females due to sexual dimorphism for TL. Prior to the ANOVAs TL data were log transformed. On average, *D. koepferae* was significantly larger than

Table 2. Average thorax length (TL) and developmental time (DT) of *D. koepferae* and *D. buzzatii* emerged in single species cultures reared in *O. sulphurea* and *T. terschekii* media. *N* = sample size, SD = standard deviation

	Males			Females		
	TL* X (SD)	DT# X (SD)	*N*	TL* X (SD)	DT# X (SD)	*N*
D. koepferae						
O. sulphurea	1.141 (0.032)	12.65 (1.059)	134	1.209 (0.032)	12.56 (1.052)	127
T. terschekii	1.12 (0.032)	12.70 (1.049)	156	1.187 (0.032)	12.59 (1.054)	144
D. buzzatii						
O. sulphurea	1.080 (0.032)	12.62 (1.076)	114	1.173 (0.032)	12.41 (1.69)	112
T. terschekii	1.075 (0.032)	12.30 (1.054)	76	1.133 (0.036)	12.19 (1.079)	72

*: in mm, #: in days.

Table 3. Analyses of variance for thorax length and developmental time in *D. buzzatii* and *D. koepferae* emerged from single species cultures grown in *O. sulphurea* or *T. terschekii* media

Source of variation	Thorax length				Developmental time		
	d.f.	SS	*F*	*p*	SS	*F*	*p*
(a) Males							
Drosophila	1	0.029	51.38	0.001	0.013	21.58	0.001
Cactus	1	0.012	35.26	0.027	1.7×10^{-3}	1.04	0.416
Drosophila × cactus	1	0.001	2.25	0.134	2.4×10^{-3}	4.12	0.043
Replicate within cactus	2	6.7×10^{-4}	0.12	0.885	3.3×10^{-3}	0.52	0.611
Vials within replicate	8	0.023	5.13	0.001	0.025	5.32	0.001
Error	442	0.25			0.03		
(b) Females							
Drosophila	1	0.044	311.71	0.001	7.2×10^{-3}	12.57	0.001
Cactus	1	2.5×10^{-3}	1.26	0.378	2×10^{-3}	0.72	0.487
Drosophila × cactus	1	4.5×10^{-4}	4.32	0.038	4.6×10^{-3}	7.93	0.005
Replicate within cactus	2	4×10^{-3}	3.60	0.077	5.7×10^{-3}	1.34	0.315
Vials within replicate	8	4.5×10^{-3}	3.98	0.001	0.017	3.72	0.001
Error	417	0.058			0.241		

D. buzzatii (Table 3). The effect of cactus hosts on TL was significant in males but not in females and the interaction *Drosophila* × cactus hosts was only significant in females (Table 3). Particularly, *D. koepferae* flies grown in cardón were significantly smaller than in *O. sulphurea* (single effect ANOVA: $F_{1,269} = 18.76$, $p < 0.001$ and $F_{1,288} = 21.13$, $p < 0.001$, for males and females, respectively). However, in *D. buzzatii* male body size was independent of the rearing conditions ($F_{1,182} = 1.54$, $p = 0.22$), whereas females reared in *O. sulphurea* were significantly larger than in cardón ($F_{1,188} = 6.44$, $p = 0.012$).

Developmental time. Mean developmental time of *D. buzzatii* and *D. koepferae* reared in both host plants are given in Table 2. Data were log transformed prior to the ANOVAs. These tests revealed that *D. buzzatii* developed faster than *D. koepferae* in both cactus media and that the interaction *Drosophila* × cactus was significant in both sexes (Table 3). Single effect ANOVAs showed that, *D. buzzatii* males developed significantly faster in cardón than in *Opuntia* ($F_{1,182} = 6.46$, $p = 0.01$) and, although, a similar pattern was observed in females, the difference was only marginally significant ($F_{1,188} = 2.90$, $p = 0.09$). On the other hand, in *D. koepferae* developmental time did not differ between cactus hosts ($F_{1,269} = 0.53$, $p = 0.47$, and $F_{1,288} = 0.17$, $p = 0.68$, for males and females, respectively).

Overall performance. Overall larval performance was assessed by means of the relative performance index (RPI = viability × TL/DT). This index was

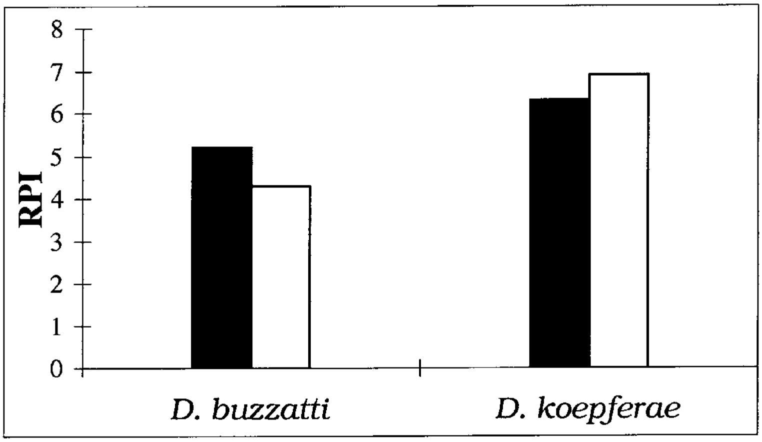

Figure 1. Relative performance index (RPI, see text for explanation) estimated for *Drosophila koepferae* and *D. buzzatii* in single species cultures grown in *O. sulphurea* (black bars) and *T. terschekii* (open bars) media.

previously used by Ruiz and Heed (1988) and modified by Krebs and Barker (1991, 1993). RPI can be considered a measure of fitness since it takes into account thorax length, which is a trait positively correlated with several adult fitness components, developmental time and viability (Heed and Mangan, 1986; Klingenberg and Spence, 1997). Figure 1 summarizes the fitness estimates for *D. buzzatii* and *D. koepferae* in both cactus hosts. The results of the ANOVA designed to analyze overall performance, with *Drosophila* and host cactus as fixed factors, showed a significant interaction effect ($F_{1,72} = 10.55$, $p = 0.002$), and significant overall differences between species ($F_{1,72} = 178.6$, $p < 0.001$). *D. koepferae* showed its best performance in cardón (RPI = 6.9), while the corresponding value in *O. sulphurea* (6.3) was slightly lower (Fig. 1). In *D. buzzatii*, overall performance was higher in *O. sulphurea* (RPI = 5.2) than in cardón (RPI = 4.3) (Fig. 1).

Mixed species experiments

First instar-larva to adult viability in mixed species cultures was high (Table 4) and within the range expected from the values estimated for both species in both cactus hosts.

The proportion of *D. buzzatii* males emerged varied not only according to the rearing medium, but also along the period of egg collection (Table 4). The proportion of *D. buzzatii* males emerged from the samples of eggs collected in the chambers on day 1 not only differed dramatically between rearing media, but also they were considerably lower than the expected 30%, particularly in cardón. However, in the next two days the proportion of *D. buzzatii* increased

Table 4. Absolute frequencies of *D. buzzatii* (Db) and *D. koepferae* (Dk) males and total number of females (fem) emerged in mixed species cultures along a three day sampling period in *Opuntia sulphurea* and *Trichocereus terschekii* media. Mean global viability (V) is also indicated for each cactus host and day

	O. sulphurea				*T. terschekii*			
	Db	Dk	Fem	V	Db	Dk	Fem	V
Day 1	58	285	327	0.64	17	316	307	0.73
Day 2	29	13	31	0.61	25	103	108	0.66
Day 3	24	11	39	0.62	28	67	87	0.65

consistently in both media, especially in *O. sulphurea* vials, in which it reached 70%, and 20–30% in cardón.

The regression analysis of the proportion of *D. buzzatii* on time revealed a significant pattern of temporal variation which was consistent across cacti (*O. sulphurea*: $F_{1,30} = 46.1, p < 0.001$ and *T. terschekii*: $F_{1,36} = 78.8, p < 0.001$).

Discussion

Our study shows that *D. buzzatii* and *D. koefperae* differ in their patterns of host plant utilization. Our first finding is that the necroses of *O. sulphurea* and the columnar *T. terschekii* can be suitable breeding sites for both species. Although there is some niche overlap, *D. buzzatii* exploits preferentially the rotting cladodes of *O. sulphurea*, while *D. koepferae* breeds on the rotting stems of cardon. The present results, indicate that evolution of host plant utilization in this pair of species had entailed a host shift accompanied by changes in oviposition preference and oviposition behavior and other life history traits such as viability, developmental time and thorax length.

According to Fogleman and Abril (1990) differential exploitation of resources may occur during two phases along the life cycle of an insect: (i) host plant selection, in which volatile compounds are used by the insect as cues to locate a suitable breeding site (Parsons, 1983), and (ii) utilization of the host plant which is the ability of the insect to use the host plant as a substrate. During the last phase, chemical factors such as nutritious quality of the substrate and the presence of toxic compounds determine the suitability of the host plant.

Field studies illustrate that *D. buzzatii* and *D. koepferae* were not differentially attracted to the necrotic tissues of the cacti present in Quilmes. However, the community of flies attracted to banana baits differed significantly from natural hosts. Krebs and Barker (1991) described, in Australia, a counterexample in which *D. buzzatii* and *D. aldrichi*, two distantly related species, were differentially attracted to three *Opuntia* hosts. However, emergence records showed that *D. buzzatii* is mainly associated with *O. sulphurea* while *T. terschekii* with cardón.

Oviposition preference, differential viability and/or interspecific competition can be invoked to account for the distinctive emergence records, that suggest differential utilization of cactus hosts during the second phase of habitat selection by Fogleman and Abril (1990).

The observed host specific viabilities in our single species experiments are in line with the patterns expected from the emergence records, i.e. *D. buzzatii* was more viable in *O. sulphurea* and *D. koeferae* in cardón. However, viability effects cannot solely explain the differences between attraction and emergence records. If we take into account the viabilities of *D. buzzatii* (58.2%) and *D. koepferae* (67.8%) in single species cultures reared in *Opuntia* medium (Table 2), the expected proportion of *D. buzzatii* in mixed cultures grown in the same cactus should have been 27%, assuming that the population of eggs oviposited in mixed chambers do not differ from the proportion of each species in the adult population. However, the expected value is higher than the proportion observed (16.9%) in the first day of egg-collection. A similar argument would show that the observed percentage of *D. buzzatii* in cardón (5.1%) is remarkably lower than the expected 22%.

Several authors have shown the importance of interspecific competition in host plant utilization in various cactophilic *Drosophila* (Benado and Montero, 1988; Krebs and Barker, 1991, 1993). Although, in the present study we did not evaluate the relative role of interspecific competition, if we assume that oviposition preference and oviposition behaviour are similar in *D. buzzatii* and *D. koepferae*, the proportions of each species in mixed cultures should have been similar along the sampling period of three days. However, the proportion of *D. buzzatii* was lower than expected in the first day in both host plants, and it increased steadily in the next two days. Furthermore, a decrease in general viability would be expected when the competing species exert negative effects on each other, however, in the present case, viability was high in mixed cultures, allowing us to argue against interspecific competition, at least for viability under the breeding conditions employed.

Thus, it can be argued that oviposition preference and differential oviposition behavior can be considered as important determinants of host plant utilization. The results in mixed species experiments suggest that *D. buzzatii* preferred *O. sulphurea* and *D. koepferae* cardón as oviposition sites. In fact, more recent studies revealed that *D. koepferae* laid more eggs in *T. terschekii* than in *O. sulphurea* (Fanara and Hasson, unpublished results).

The temporal patterns observed in mixed species cultures can be interpreted as differential oviposition behavior. On one hand, *D. koepferae* females laid most of their eggs the first day collection, especially when the host was cardón and, decreasing its proportion afterwards. On the other hand, the proportion of *D. buzzatii* increased steadily after the first day of egg collection, particularly in *O. sulphurea*.

Differential viability and oviposition preference in *D. buzzatii* and *D. koepferae* can be related to several aspects of the host plants. One of them is related to the chemical composition of the host cacti. Kircher (1982) reported that columnar and *Opuntia* cacti of the Sonoran desert differ in their chemical compositions. On one hand, opuntias have, in general, a higher water content and also significant levels of free sugars, whereas columnars are chemically more complex due to an unusual content of lipids and triterpene glycosides, which were shown to be toxic for some *Drosophila* species. These disparate compositions may have important consequences in the community of microorganisms present in the necroses of *Opuntia* and columnars, providing nutrients to the growing larvae. In fact, Starmer (1981) have shown that these cacti are quite different when physiological properties of the yeast communities are taken into account, emphasising that they clearly represent distinctive habitats for *Drosophila*.

Further biological differences between cactus hosts such as their spatial and temporal availability may be related to our observations of different oviposition strategies. In natural communities host plant density and size are negatively correlated and Thompson and Pellmyr (1991) have suggested that this may lead to the evolution of different oviposition strategies. Cardón rots are relatively less abundant and less ephemeral than *Opuntia* substrates (Heed and Mangan, 1986; Etges, 1993, and field observations). Thus, according to Heed and Mangan (1986) large stemmed cacti, like *T. terschekii*, may offer a more stable (predictable) habitat for larvae than do small stemmed cacti and less predictable for adults searching for new feeding and oviposition sites. Likewise, under these circumstances relatively more reproductive effort is found in species which use large stemmed cacti, in line with the differences detected in our mixed species experiments. Thus, it can be argued that utilization of a long lasting and patchely distributed host plant, like cardón, may have driven life history evolution in *D. koepferae* towards a massive reproductive output upon location of a new breeding site. Contrarily, *D. buzzatii's* strategy may have arisen as an adaptation to avoid crowding in ephemerous and spatially abundant resources. Such contrasting patterns of trophic predictability are also concordant with the longer developmental times found in *D. koepferae*, whereas in *Opuntia* rots fast developing flies, such as *D. buzzatii* would be favored. Similarly, the large size of *D. koepferae* seems to be an expected outcome in a species breeding in spatially unpredictable resources and thus, selected for greater dispersal ability (Heed and Mangan, 1986; Norry, 1995).

Viability data give support to Jaenike's (1978) theory of optimal oviposition behavior since both species tended to lay their eggs in the host plant where their progeny's viability is increased. Yet, thorax length and developmental time data are not unequivocal, since larger *D. koepferae* flies emerged in cultures with its unpreferred host, and *D. buzzatii* developed faster in cardón rather than in *O. sulphurea*. However, when we considered the three life history traits in a more inclusive index of overall performance, the estimates for both species are in agreement with theoretical expectations.

Life history traits may be useful in determining the causes of the community structure in Quilmes since major trends could be inferred for both species. However, the relative performance index do not tell us the entire story, since it does not provide a causal explanation for the greater evolutionary success of *D. buzzatii*. Our present data suggest that oviposition preference and oviposition behavior may lay at the basis of this question.

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