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Oviposition and performance in natural hosts in cactophilic *Drosophila*

Eduardo M. Soto · Julieta Goenaga · Juan P. Hurtado · Esteban Hasson

Abstract The cactus–yeast–*Drosophila* model system provides an excellent opportunity to investigate the significance of ecological factors in evolution. *D. buzzatii* and *D. koepferae* are sister species, with partially overlapping distribution ranges and a certain degree of habitat overlap. The main breeding and feeding resources of *D. buzzatii* are the decaying cladodes of prickly pears (genus *Opuntia*), whereas *D. koepferae* utilizes mainly columnar cacti of the genera *Cereus* and *Echinopsis*. These host plants differ in their chemical composition, the microflora associated to the decaying process and patterns of spatial and temporal predictability. The aim of the present work is to investigate host plant selection and utilization of two different cactus hosts. We report the results of field and laboratory studies examining behavioral traits related to egg-laying (oviposition preference and host acceptance) and several measures of performance (viability, developmental time, wing morphology and starvation resistance) in flies reared in the two main host cacti that *D. buzzatii* and *D. koepferae* exploit in the studied area: *O. sulphurea* and *E. terschekii*. The main conclusion of our study is the clear connection between the distribution of the cactophilic species, *D. buzzatii and D. koepferae,* and the abundance of the two main natural hosts, which is line with the remarkable influence that cactus hosts impose on larval and adult life history traits and behavioral traits. Overall, the results of field and laboratory work point to the important role of host plant shifts in the evolutionary history of these species.

Keywords Habitat selection · Life history traits · Natural breeding resource · Optimal oviposition behaviour · Cactophilic *Drosophila*

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Introduction

The interest in the role of ecology in speciation developed from the idea that the access to new ecological resources may promote diversification (Schluter 2001). Ecological speciation should not be considered as synonymous with sympatric speciation, since divergent selection can occur either in sympatry or allopatry (Funk et al. 2006). However, the role of ecology in speciation has only recently started to be systematically evaluated (Schluter 2001; Drès and Mallet 2002; Dieckmann et al. 2004). Studies involving diverse groups (as angiosperms, fishes, frogs, birds, pigeons and, mainly, insects -mostly butterflies and fruit flies-) revealed a link between ecological divergence and reproductive isolation (Funk et al. 2006). In particular, changes in habitat or diet are positively associated with reproductive isolation (Funk et al. 2006; Rova and Björklund 2011), giving support to the idea that host plant shifts may be a plausible explanation for the remarkable diversity in phytophagous insects.

The majority of herbivores develop to maturity in close association with the host plant, which represents their immediate habitat. The insect-host association persists into the adult stage in many groups. So, acquisition of a novel host may drive adaptive divergence in traits related to the location of, mating, oviposition and performance in a new host (Mitter and Futuyma 1983; Etges 1990; Jaenike 1990; Fanara and Hasson 2001; Matzkin et al. 2006; Jaureguy and Etges 2007; McBride 2007; Tilmon 2008). In addition, adaptation to new hosts may cause the evolution of extrinsic and/or intrinsic habitat isolation, highlighting the evolutionary role of host plant shifts (e.g. Etges et al. 2006; see also Coyne and Orr 2004 for a review).

Differential exploitation of alternative resources may occur during two phases along the life cycle of an insect: (1) host plant selection, in which volatile compounds are used by the insect as cues to locate a suitable breeding site, and (2) utilization of the host plant which is the ability of the insect to use the host plant as feeding substrate (Fogleman and Abril 1990). Host plant selection is the search for, movement to and settling on a potential breeding site and is followed by the acceptance or rejection of the site for oviposition. Female insects use a wide variety of cues to evaluate potential sites for oviposition, either abiotic like environmental light (Wogaman and Seiger 1983), temperature (Fogleman 1979), chemical composition (Amlou et al. 1998; Fanara and Hasson 2001), resource texture and/or biotic, such as the presence of predators, conspecifics and heterospecific larvae in the resource (Chess and Ringo 1985). However, to understand the role of host shifts in speciation we need to evaluate how host-related recognition and performance traits change through time (Bush 1975; Coyne and Orr 2004).

The ability to utilize different breeding sites (performance) can be assessed by measuring life history traits (Thompson 1988). Survival, growth rate and fecundity are directly related to fitness whereas body size, desiccation resistance, starvation resistance and developmental rate are indirectly related to fitness. Life history traits may be involved in trade-offs with other traits that may constraint adaptive evolution (Stearns 1992; Curtsinger et al. 1994; Roff 1996, 1997, 2000; Cortese et al. 2002; Mayhew 2006). In addition, the relationship between fitness and life history traits as well as among life history traits themselves may change across environments, adding another dimension to an a priori complex issue. Also, nutritional characteristics of alternative resources may have a strong influence on life history traits (Gebhardt and Stearns 1988, 1993; Markow and O'Grady 2005).

Although the cost of egg production may be compensatory relative to the costs of other physiological and morphological costs, the ability to choose a site for egg-laying may

critically affect female's fitness (Partridge et al. 1987; Chapman et al. 1998; Yanagi and Miyatake 2003; Berenbaum and Feeny 2008; Yang et al. 2008), particularly, in insects in which larvae are confined to the resource chosen by their mothers. Therefore, natural selection should favor females exhibiting a preference for egg-laying sites in which offspring fitness is maximized (Singer 1972; Jaenike 1978), a line of reasoning that has been termed the optimal oviposition behavior hypothesis (Jaenike 1978) or the preference-performance hypothesis (Craig and Itami 2008; Gripenberg et al. 2010). Current evidence for a positive relationship between preference and performance remains ambiguous (Rausher 1979; Underwood 1994; Faria and Fernandes 2001). However, proponents of the feeding niche constraints hypothesis argued that the kind of resource variation an insect encounters influences the evolution of the preference-performance relationship (Craig and Itami 2008). In fact, the authors hypothesize that insects share certain fundamental constrains that limit their ability to respond to this variations and influence the evolution of the preference-performance relationship. A prediction of this hypothesis is that insects with similar feeding niches will evolve similar preference-performance relationships (Craig and Itami 2008).

The cactus–yeast–*Drosophila* model system provides an excellent opportunity to investigate the significance of ecological factors in evolution (Barker 1982; Fogleman 1982; Starmer 1982; Barker and Starmer 1999; Markow and O'Grady 2008; Hasson et al. 2009). The South American *D. buzzatii* Patterson and Wheeler and its sibling *D. koepferae* Fontdevila and Wasserman are two closely related species of the *D. buzzatii* complex (*repleta* group, *mulleri* subgroup; Ruiz and Wasserman 1993), that have partially overlapping ranges in the arid lands of Argentina, (Fontdevila et al. 1988; Hasson et al. 1992). Although there is a certain degree of habitat overlap, the main breeding resources of *D. buzzatii* are the decaying cladodes of prickly pears (genus *Opuntia*), while *D. koepferae* mainly utilizes columnar cacti of the genera *Cereus* and *Echinopsis* [formerly *Trichocereus*] and *Neocardenasis* (Fontdevila et al. 1988; Hasson et al. 1992, 2009). Prickly pears and columnars exhibit dramatic differences in chemical composition (Kircher 1982; Fogleman and Abril 1990), in the microflora associated to the decaying process (Starmer et al. 1990) and in spatial abundance and temporal availability (Etges 1993; Breitmeyer and Markov 1998; Fanara et al. 1999). Previous studies have shown that *D. buzzatii* and *D. koepferae* optimize certain aspects of performance in their respective primary hosts (Soto et al. 2008a, b).

In this paper, we report the results of field and laboratory studies aimed to investigate host selection and utilization of two different cactus hosts. In particular, we investigate oviposition preference and host acceptance for *O. sulphurea* Gillies ex Salm-Dyck and *E. terschekii* (Britton & Rose) Friedrich & Rowley) and viability, developmental time, wing morphology and starvation resistance as measures of performance. Our null hypotheses are that both species do not differ in preference for natural resources and performance when reared in different host plants. Alternatively, we may hypothesize that *D. koepferae* and *D. buzzatii* are preferentially attracted to, prefer to lay eggs on and perform better in each species' primary host in agreement with the expectations of the preference-performance hypothesis.

Materials and methods

Fly collections and stock maintenance

Field experiments and fly collections were carried out close to San Agustín del Valle Fértil (30.3°S 67.3°W, San Juan Province, Argentina) in March 2006. Two cactus species, the

prickly pear *O. sulphurea* Gillies ex Salm-Dyck and the columnar *E. terschekii* (Britton and Rose) Friedrich and Rowley are the most abundant potential hosts in the studied area. Flies were collected by net sweeping on fermented banana baits, sexed upon arrival to the laboratory and used to generate isofemale lines (lines hereafter) by placing individual females in vials containing 5 ml of Instant *Drosophila* Medium (Carolina Biological Supplies). Since females of these species are morphologically indistinguishable, lines were identified by examining the genitalia (aedeagus) of several progeny males of each line (Vilela 1983). After five generations in the laboratory, thirty lines of each species were randomly chosen to generate two outbred stocks, one of each species, by mixing 20 males and 20 females of each isofemale line. Outbred stocks were reared under identical conditions for five generations before the experiments.

We also collected rotting and fresh materials of *O. sulphurea* and *E. terschekii* for the preparation of two types of 'semi-natural' media. Fresh materials were stored at −20°C and necrotic stems of *E. terschekii* and cladodes of *O. sulphurea* were maintained by adding 10 g of fresh material every two weeks to the necroses collected in the field. For the preparation of 'semi-natural' media, pieces of fresh cactus of each species were ground in a blender and 10 ml of the liquefied cactus plus 1 g of agar–agar were poured into standard *Drosophila* vials, for the measurement of life history traits, or in small plates for the assessment of host acceptance and oviposition preference (see below). Vials and plates were autoclaved and, after cooling, 0.1 ml of the fermenting juice of the corresponding cactus were inoculated into each vial and incubated at 25°C for 24 h before the experiments. Both types of experimental media exhibited similar consistency.

Field study

The main objective of the field study was to investigate habitat selection in nature, in a collecting site where *D. koepferae* Fontdevila and Wasserman and *D. buzzatii* Patterson and Wheeler coexist in a two cactus environment. We examined the distribution and biomass of *O. sulphurea* and *E. terschekii* throughout the sampling area. A priori, we distinguished two different sectors separated by a local road (Fig. 1). These Sectors were defined according to field observations and took into account geographical features. The first sector is close to a river bed and the second is at the foot of one of the hill ranges that enclose the valley. The first one is more humid and the vegetation more dense than in the other (field observations). In the studied area, we delineated 20 transects (ten at each side of the road). Each transect was 30 m long and 3 m wide and were separated by 10 m. Along each transect we counted the number of individuals of each cactus species and, for each cactus, we measured height and average size of arms or cladodes (in meters). With this information plus the data of the weight of pieces of fresh material collected in the same area, we estimated the biomass (in g per m^2) of *O. sulphurea* and *E. terschekii*. These data were analyzed by means of a two-way ANOVA, with Sector and Resource as fixed factors.

Subsequently to the characterization of the experimental area, we prepared, in situ, 30 baits that were randomly distributed in the area. Fifteen baits were made with artificially fermented pieces of fresh *O. sulphurea* and the remaining with artificially fermented *E. terschekii*. Each bait consisted of a transparent plastic box (15 cm × 10 cm × 8 cm) containing about 200 g of fresh cactus materials of *O. sulphurea* or *E. terschekii* fermented with 5 ml of the exudates from naturally occurring rots of the corresponding cactus species. After 48 h, all flies that were in the baits were recovered using an entomological net and the boxes covered with plastic lids. To estimate the species ratio in the samples of flies
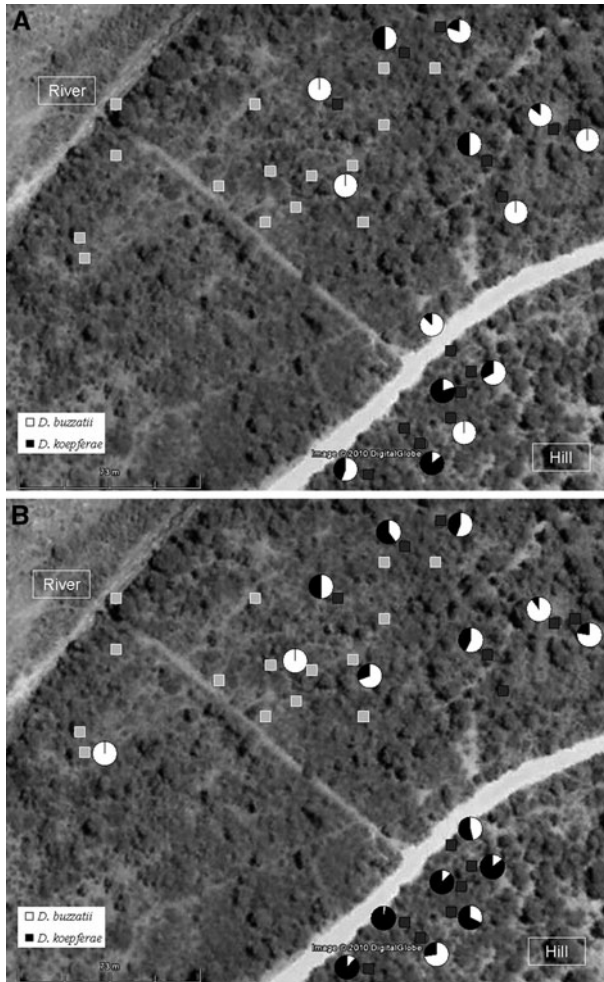
Fig. 1 Proportion of *D. buzzatii* (*white*) and *D. koepferae* (*black*) in captured (**a**) and emerged flies (**b**), in traps with *O. sulphurea* (*gray square*) o *E. terschekii* (*black square*)

recovered from the baits we decided to use males, which can be identified by means of the inspection of the genitalia (Soto et al. 2007). The use of this methodology has the underlying assumption that fly attraction to the baits is independent of sex. We did not use females because *D. koepferae* and *D. buzzatii* females are morphologically indistinguishable and can only be differentiated by means of the analysis of the progeny of individual gravid females. However, this methodology could introduce a distortion since *D. buzzatii* females are more prone to lay eggs than *D. koepferae* under laboratory conditions to estimate the species ratio. All traps were recovered and transported to the laboratory and kept at 25 ± 1°C and 12:12 light/dark photoperiod. All flies that emerged in the traps were aspirated off daily and identified, as described above, to estimate the species ratio.

Laboratory assays

Behavioral traits: oviposition preference and acceptance of natural host

For the assays of oviposition preference and host acceptance, 20 pairs of sexually mature flies (4–5 days old) were released in chambers with 6 plates containing a 'semi-natural' medium prepared with fermented *O. sulphurea* or *E. terschekii*. In oviposition preference assays, two different resources were presented to the flies consisting of 3 plates with *O. sulphurea* and 3 with *E. terschekii*. Oviposition preference was estimated as the proportion of eggs laid in *O. sulphurea*. To analyze oviposition preference data we utilized a one-way ANOVA with *Drosophila* species (fixed) as the only source of variation. An angular (arcsine square root) transformation was applied prior to statistical analysis.

The host acceptance study, unlike the preference assay, consisted of non-choice experiments in which the 6 plates in each chamber contained only one type of resource (*O. sulphurea* or *E. terschekii*). Five replicated chambers were run for each combination of cactus and *Drosophila* species. After 48 h we counted the number of eggs in each plate, and host acceptance was estimated as the number of eggs in each chamber averaged across plates. These data were analyzed by means of a two-way ANOVA, with *Drosophila* species (D) and resource (cactus, R) as fixed factors. In this case, a logarithmic transformation was applied to the data prior to statistical analysis.

Performance traits

To measure performance traits, 100 pairs of sexually mature flies (4–5 days old) were released in egg-collecting chambers. Eggs were allowed to hatch and batches of 30 first-instar larvae were transferred to vials containing a 'semi-natural' medium prepared with fermented *O. sulphurea* or *E. terschekii*.

Vials were incubated at $25 \pm 1°C$ and a 12:12 light/dark photoperiod until the emergence of flies that were also maintained under the same thermal and photoperiod regimes.

Larval traits

Larval viability was estimated as the proportion of adults emerged relative to the number of larvae seeded in each vial. An angular transformation was employed prior to statistical analysis.

To estimate developmental time we measured the time (in hours) elapsed since the transfer of first instar larvae to the vials until adult emergence. We applied a logarithmic transformation to developmental time data prior to statistical analysis. Both larval traits were analyzed by means of two-way ANOVAs with *Drosophila* species and resource as fixed factors. Larval viability and developmental time were measured in eleven replicated vials run for each combination of species and cactus.

Adult traits

Starvation resistance was measured in flies reared in each cactus and fed with instant *Drosophila* medium before exposure to the starvation diet (Experiment A). We also measured starvation resistance in flies grown in vials containing Instant *Drosophila* Medium and fed as adults with *O. sulphurea* or *E. terschekii* before exposure to the starvation diet (Experiment B). Starvation resistance was estimated as the time elapsed (in

hours) from the moment in which flies were exposed to a starvation diet until death. Starvation diet consisted of 5 ml of 1.7% agar in water that provided moisture but not food in standard culture vials. Groups of seven, 2–7 day old and virgins, flies of the same sex were transferred to the vials in which starvation resistance was assessed. For each combination of *Drosophila* species, sex and resource, we run twenty five and twenty replicated vials for experiments *A* and *B*, respectively. All vials were incubated at a constant temperature of 25°C under a 12 h light/dark cycle. Survival was scored daily at 800, 1400 and 2000 hours, until the death of all flies. Scores of starvation resistance for each individual fly were used to obtain the median survival time per vial, which was the variable considered in statistical analyses. We employed an ANOVA with *Drosophila* species, resource and sex as fixed factors to analyze starvation resistance. In addition, we also adopted an analytical demographic approach to investigate mortality differences among treatments based on Gompertz function (Carey 2001). The analysis of mortality curves often bring more information than standard analysis based on comparisons among means, since these techniques offer the possibility to discriminate populations that, despite sharing a mean lifespan, may differ in initial mortality rate and/or increase of mortality with age. For this analysis replicates within treatments were pooled. Ninety five percent confidence intervals of the functions parameters (initial mortality rate and increase of mortality with age) were obtained by means of an optimization procedure that was performed using the package Statistica (StatSoft Inc. 2001).

We used a geometric morphometric approach to analyze wing size. Three males and three females emerged in each vial were randomly chosen and both wings of each individual removed, mounted on a slide and photographed. Wing images were captured with a digital camera attached to a binocular microscope and connected to a computer. We defined nine landmarks on the ventral face of each wing (Soto et al. 2011) and recorded using TPS DIG (Rohlf 2001 v. 1.31, available at http://life.bio.sunysb.edu/morph/). The centroid size, calculated by taking the square root of the sum of the squared distances between each landmark and the centroid of each wing (Dryden and Mardia 1998), was used as an estimate of wing size. We utilized the same ANOVA design, described above for starvation resistance, to partition phenotypic variance into sources of variation attributable to *Drosophila* species (D), resource (R) and sex (S), all factors were set fixed. Wing size was measured in eleven replicated vials run for each combination of species and cactus.

All statistical analyses were performed using GLM implemented in the STATISTICA 6.0 software package (StatSoft Inc. 2001).

Results

Host selection and host use: the field study

The field survey revealed *O. sulphurea* and *E. terschekii* are not uniformly distributed throughout the sampling area ($F_{1, 20} = 11.83$, $P < 0.01$). The latter was more abundant in the Hill than in the River sector ($F_{1, 20} = 14.82$, $P < 0.001$) whereas the biomass of *O. sulphurea* did not differ between areas ($F_{1, 20} = 0.02$, $P = 0.914$). Finally, the comparison of the abundance of both resources in the different sectors, showed that, in the Hill sector, *E. terschekii* was more abundant than *O. sulphurea* ($F_{1, 20} = 20.59$, $P < 0.001$) and that in the River sector both types of resources were equally abundant ($F_{1, 20} = 1.28$, $P = 0.271$).

Attraction to natural resources

Baits prepared with *O. sulphurea* were less effective than *E. terschekii*, only five females and one *D. buzzatii* male could be recovered from the complete set of *O. sulphurea* baits. On the contrary, we were able to recover 267 flies (161 females and 86 males) from 13 out of the 15 *E. terschekii* baits in a proportion of 7 *D. buzzatii*: 3 *D. koepferae* (Fig. 1a).

Subsequently, all baits were transported to the laboratory and maintained until adult flies stopped to emerge. A total of 166 flies and 1,128 emerged from 3 *O. sulphurea* and 14 *E. terschekii* baits, respectively. The ratios of *D. buzzatii* to *D. koepferae* (species ratio) in flies emerged from *O. sulphurea* and *E. terschekii* were 9:1 and 4:6, respectively, which according to the ANOVA were significantly different ($F_{1, 14} = 10.23$, $P < 0.01$) (Fig. 1b).

Comparisons between flies attracted to and emerged from each type of cactus showed that the proportion of *D. buzzatii* was greater in flies attracted to than in flies emerged from the baits ($F_{1, 24} = 7.19$, $P < 0.05$). However, given the observed differences in biomass of each type of cactus between sectors, we also examined if the species ratio also varied between the River and Hill sectors in collected and emerged flies. The corresponding ANOVA showed that the species ratio in flies collected in baits did not differ between sectors ($F_{1, 11} = 2.06$, $P = 0.18$), while differences between sectors were significant in emerged flies. *D. buzzatii* was more abundant in the River sector (species ratio 6:4) than in the Hill sector (species ratio 3:7).

Laboratory assays

Behavioral traits: oviposition preference and host acceptance

The ANOVA for host acceptance showed that *D. koepferae* laid more eggs than *D. buzzatii*, irrespective of the substrate ($F_{1, 16} = 91.34$, $P < 0.001$) (Fig. 2a), while the cactus host ($F_{1, 16} = 0.92$, $P = 0.35$) and the *Drosophila* species by Resource interaction were not significant ($F_{1, 16} = 1.06$, $P = 0.319$).

Oviposition preference assays revealed significant differences between species in the proportion of eggs in each cactus ($F_{1, 17} = 6.59$, $P < 0.05$). A posteriori comparisons showed that the proportion of eggs laid by *D. buzzatii* in *O. sulphurea* ($\cong 70\%$ eggs) was
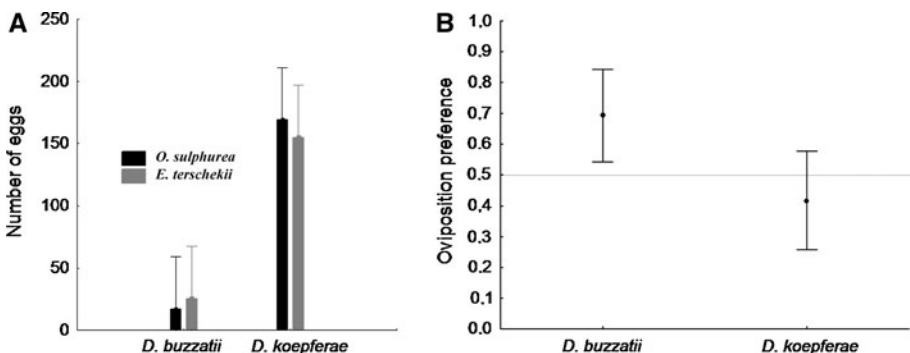

Fig. 2 Means values of **a** Host acceptance (number of eggs laid) and **b** oviposition preference (as the proportion of eggs laid in *O. sulphurea*) of *Drosophila buzzatii* and *D. koepferae* for their natural cactus hosts

Table 1 Results of the ANOVAs for larval viability (LV) and developmental time (DT) in *D. buzzatii* and *D. koepferae* reared in vials with media prepared with *O. sulphurea* or *E. terschekii*

Source of variance	g. l.	LV		DT	
		MS	*F*	MS	*F*
Drosophila species (D)	1	0.000	0.013	0.000	0.051
Resources (H)	1	0.237	9.451**	0.000	0.057
D × H	1	0.024	0.968	0.022	11.504**
Error	40	0.025		0.002	

** $P < 0.01$

significantly larger than in *E. terschekii*, whereas differences between cactus hosts were not significant in *D. koepferae* ($\cong 42\%$ of the eggs laid in *O. sulphurea*) (Fig. 2b).

Performance traits

Larval traits. We investigated larval viability and developmental time as proxies of larval performance. Differences between species for larval viability were not significant and mean larval viability was greater in *O. sulphurea* ($\cong 80\%$) than in *E. terschekii* ($\cong 68\%$) in both species (Table 1; Fig. 3a).

Concerning developmental time, differences between species, between flies reared in different resources and the *Drosophila* species by Resource interaction were significant (Table 1). *Drosophila buzzatii* developed faster in *O. sulphurea* (298.7 h) than in *E. terschekii* (313.3 h) and the reverse was true for *D. koepferae* (developmental time: 313.2 h in *O. sulphurea* and 299.2 h in *E. terschekii*) (Fig. 3b).

Adult traits. The effect of the rearing cactus on adult performance was investigated by means of the assessment of starvation resistance and wing size in flies reared in *O. sulphurea* or *E. terschekii*. The ANOVA for starvation resistance revealed significant differences between species, between flies reared in different resources and between sexes (Table 2). On average, flies reared in *O. sulphurea* live longer than flies reared in *E. terschekii*, *D. buzzatii* outlived its sibling irrespective of the cactus host and females (*D. buzzatii*: 190.4 h; *D. koepferae*: 100.7 h) outlived males (*D. buzzatii*: 147 h; *D. koepferae*: 70.7 h) regardless of the cactus host and the species (Table 2). All double interactions were also significant (Table 2). Concerning the *Drosophila* species by Sex interaction further analysis showed that differences between males and females in starvation resistance, measured as the ratio of the trait mean in females and males (a measure of the sexual dimorphism: SD) were larger in *D. koepferae* (SD = 1.42) than in *D. buzzatii* (SD = 1.29) (Tukey post hoc comparisons $F_{1, 235} = 20.93$, $P < 0.001$). Regarding the Resource by Sex interaction, the sexual dimorphism was more pronounced in flies reared in *E. terschekii* (Tukey post-hoc comparisons $F_{1, 235} = 20.93$, $P < 0.001$). We also explored the results of starvation resistance assays using Gompertz analytical demographic procedure. The analyses of mortality curves (Fig. 3c) showed that regressions of mortality as a function of time were significant for all treatments (not shown). Moreover, tests of parallelism revealed significant differences among treatments in both species (*D. buzzatii*: $F_{3, 141} = 5.34$, $P < 0.01$; *D. koepferae*: $F_{3, 99} = 5.38$, $P < 0.01$). Further analysis showed that differences in initial mortality rates among groups were mostly responsible for the
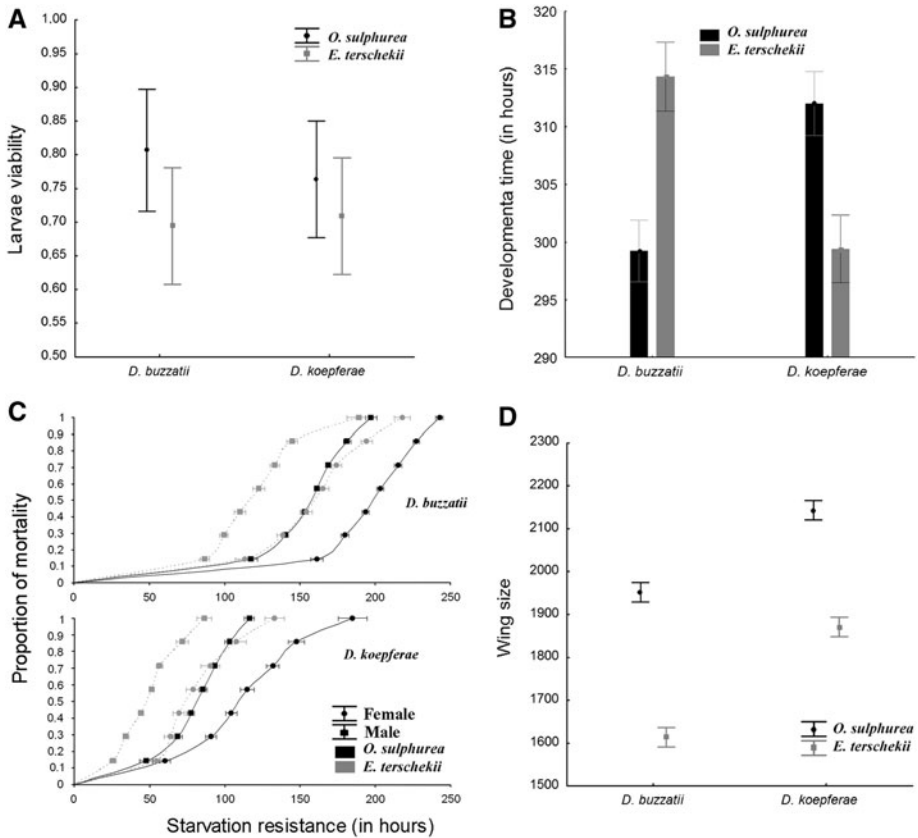
Fig. 3 Mean values of performance traits **a** Larval viability; **b** Developmental time (in hours); **c** Mortality as a function of time (see text for explanation) and **d** Wing size in *Drosophila buzzatii* and *D. koepferae* reared in *O. sulphurea* (*black*) and *E. terschekii* (*gray*). *Bars* correspond to standard deviations

significance of tests of parallelism rather than to differences in the rate of increase of mortality with age (results not shown).

We also studied the effect of the cactus host on starvation resistance in flies reared in laboratory medium and fed with *O. sulphurea* or *E. terschekii* before exposure to the starvation diet. On average, starvation resistance was about three times lower in these flies than in flies that grew up in cactus media during larval life. The ANOVA showed, as in the experiment described above, that *D. buzzatii* was more resistant to food shortage than *D. koepferae* and that females were more resistant than males (Table 2). The observation that the sexual dimorphism was more apparent in flies reared in *E. terschekii* (Tukey comparisons, $F_{1,\ 125} = 21.96$, $P < 0.001$) than in *O. sulphurea* can account for the significant Resource by Sex interaction ($F_{1,\ 235} = 0.374$, $P = 0.542$).

Concerning wing size, females had significantly larger wings than males (the usual direction of the sexual dimorphism observed in *Drosophila* for size related traits), flies reared in *O. sulphurea* were larger than in *E. terschekii* and *D. koepferae* had larger wings than *D. buzzatii* (Table 2). Finally, wing size responded in different manners to the rearing medium, as suggested by the significant *Drosophila* species by Resource interaction

Table 2 Analyzes of variance for starvation resistance (SR) and wing size (WS) in *D. buzzatii* and *D. koepferae* reared in vials containing media prepared with *O. sulphurea* or *E. terschekii*)

Source of variance	SR (Experiment A)			SR (Experiment B)			WS		
	g.l.	CM	F	g.l.	MS	F	g.l.	MS	F
Drosophila species (D)	1	27.071	1,350.3***	1	8.49	21.0***	1	3,303,752.07	378.279***
Resource (R)	1	6.314	314.9***	1	0.17	0.42	1	6,137,251.78	702.715***
Sex (S)	1	5.123	255.5***	1	43.79	108.48***	1	735,255.46	84.187***
D × R	1	0.495	24.7***	1	0.06	0.14	1	71,229.87	8.156**
D × S	1	0.088	4.4*	1	1.52	3.77	1	11,965.27	1.370
R × S	1	0.140	6.9**	1	8.25	20.40***	1	18,470.80	2.115
D × R × S	1	0.009	0.5	1	11.49	3.68	1	1,356.22	0.155
Error	235	0.020		917	0.40		255	8,733.63	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

(Table 2). In fact, differences between flies reared in different resources were more pronounced in *D. buzzatii* than in *D. koepferae* (Fig. 3d).

Discussion

The first conclusion of our study is the clear connection between the distribution of the cactophilic species, *D. buzzatii* and *D. koepferae,* and the abundance of the two main natural hosts, which is line with the remarkable influence that cactus hosts impose on larval and adult life history traits and behavioral traits.

The species composition of our samples of flies attracted to natural substrates may be considered as representative of the first phase of host plant selection and that of flies emerged from natural substrates as the outcome of the differential ability of flies to use a host plant as rearing substrate. During the first phase, volatile compounds produced by the decaying cactus tissues are used by the insect as cues to locate a suitable substrate either for feeding and/or egg-laying, and other chemical factors related to nutritious quality and the presence/absence of toxic compounds are the main determinants of the composition of the community of flies emerging from the substrates (Fogleman and Abril 1990). Thus, differential utilization of a host plant may be the outcome of interspecific variation in egg-laying behavior, differential viability and/or differential competitive ability (either intra and/or interspecific).

Our field results show that the proportions of *D. buzzatii* and *D. koepferae* in flies emerged from the baits prepared with natural substrates vary accordingly with the relative abundance of *O. sulphurea* and *E. terschekii* that vary across the studied area. This means that *D. koepferae* was more abundant in the Hill sector where *E. terschekii* dominates, whereas in the River sector, with roughly equal abundance of both cactus species, the abundance of *D. koepferae* decreases. However, it should be noted that the estimation of the species ratio in samples of flies collected in field baits is based on the assumptions that attraction to the baits is independent of sex and also that the sex ratios are 1/1 for both species. Actually, if species differ in their sex ratios and mating strategies, the outcome of our study could be affected. Sex ratio in flies attracted to the cactus baits was skewed in the

direction of males, which is in sharp contrast with the 1:1 sex ratio in simultaneous collections using fermented banana (results not shown). The main difference between collections with cactus and banana baits was that the former were exposed to the flies for a longer period (24 h) while collections on banana baits were accomplished in a couple of hours. A likely explanation for such difference may be that males dispersed soon after feeding and mating in cactus baits, whereas collections on banana baits give a snapshot of attracted flies. Nevertheless, species-specific genetic markers or subtle differences in internal anatomy between *D. buzzatii* and *D. koepferae* will surely provide the tools to investigate the validation of the assumptions.

However, the proportions of *D. buzzatii* and *D. koepferae* attracted to natural breeding sites may not be representative of the species composition of the population of eggs entering cactus-rotting pockets. Perhaps flies attracted to *O. sulphurea* and *E. terschekii* settle on the necrotic tissues just for feeding and/or mating and not necessarily for egg-laying. In fact, simultaneous collections showed that both species are equally attracted to fermented banana baits (Fanara et al. 2006, results not shown), an observation that differs from the species ratio (7 *D. buzzatii* : 3 *D. koepferae*) attracted to cactus baits. A qualitatively similar situation has been reported in Northwestern Argentina, where the proportion of *D. buzzatii* attracted to cactus necroses was higher than to banana baits (Fanara et al. 1999). Overall, field results suggest that *D. buzzatii* may be endowed with a sensory system more capable of perceiving the subtleties of the cactophilic habitat than its sibling (see below).

In summary, field results indicate (1) that cactus baits provide suitable substrates for feeding and oviposition; (2) *D. buzzatii* represented 90% of the flies emerged from *Opuntia* baits confirming its description as a prickly pear dweller (Hasson et al. 2009) and (3) *D. koepferae* can be characterized as the resident species in *E. terschekii*, given the increment of its proportion in flies emerged from relative to flies attracted to *E. terschekii* baits.

The difference in the species ratio between attracted and emerged flies may be accounted for differences in egg-laying behaviour. Host acceptance assays revealed that both types of cactus were equally accepted by *D. buzzatii* and *D. koepferae* as egg-laying site regardless of fecundity differences between species. In contrast, oviposition preference assays revealed that *D. buzzatii* prefers its primary host as egg-laying site, whereas *D. koepferae* laid similar quantities of eggs in *E. terschekii* and *O. sulphurea*. These results are in line with our field data of attraction to and emergence from natural substrates, suggesting once again that *D. buzzatii* has a more highly developed sensory system capable of telling apart primary from secondary hosts for egg-laying.

Most cactophilic *Drosophila* living in desertic regions face extreme thermal conditions and limited humidity (Marron et al. 2003), and, particularly relevant are the temporal and spatial availability of feeding and breeding resources throughout the year. On the one hand, *Opuntia* sp. offers an ephemeral but spatially predictable resource (primarily during the rainy season). On the other hand, rotting pockets of *Echinopsis* sp. offers long-lasting resources that are spatially less predictable (Hasson et al. 2009). Such differences in temporal and spatial patterns of resource availability may impose dissimilar selective pressures on traits related to the ability of searching new feeding sites and to tolerate periods of food shortage. We further investigated resource utilization by measuring larval viability and rate of development in flies grown in the two cactus hosts. These experiments showed that *D. buzzatii* achieved its best performance (greater larval viability and shorter developmental time) in *O. sulphurea*, its preferred host. These results may be interpreted as the outcome of evolutionary adaptive responses to the use of *O. sulphurea* that represent an ephemeral rearing substrate (Fanara et al. 1999). Interestingly, *D. koepferae* also achieved

its best performance, in terms of larval viability in *O. sulphurea* and maximized developmental rate in *E. terschekii*. We also evaluated the effect of the cactus host in adult flies by measuring wing size, which is correlated with dispersal ability, and starvation resistance. Overall, wing size data revealed general trends that are coincident with previous studies: females, flies reared in *O. sulphurea* and *D. koepferae* have larger wings than males; flies emerged in *E. terschekii* and *D. buzzatii* (reviewed in Hasson et al. 2009).

The relationship between starvation resistance and the rearing substrate has not been addressed in *Drosophila*, probably because most model species have an intractable ecology. Our results show that the larval rearing substrate has a paramount effect on starvation resistance, flies reared in *O. sulphurea* outlived flies grown in *E. terschekii*, *D. buzzatii* lived twice as much as *D. koepferae* and females tended to outlive males under starvation conditions. Interestingly, the cactus host effect was enough to partially overthrow the sexual dimorphism, as illustrated by the fact that males reared in *O. sulphurea* lived as long as females reared in *E. terschekii* in both species.

In *Drosophila*, lipids and carbohydrates reserves are the main energy source for resistance to starvation and desiccation (Marron et al. 2003). In this sense, it is interesting to note that *Opuntia* sp. have larger contents of free sugars and lipids [cholesterol, sitosterol and ω-3 fatty acids] (Stintzing and Carle 2005) than columnar cacti, which have a complex chemistry that includes the presence of toxic compounds such as alkaloids, atypical fatty acids and triterpenes (Kircher 1982; Fogleman and Abril 1990; Starmer et al. 1990; Stintzing and Carle 2005). Thus, the presence of alkaloids in columnar cacti and the rich nutritional environment offered by prickly pears to the growing larvae may lay at the basis of the effects that cactus hosts have on several aspects of the biology of cactophilic *Drosophila* (Hasson et al. 2009). Furthermore, such chemical differences suggest that a host shift may be easier for *D. koepferae* than for *D. buzzatii* since it may imply a shift from a 'hostile' environment, as *E. terschekii*, to a more benign environment, as *O. sulphurea*. In fact, preliminary studies have shown that increasing doses of the alkaloid fraction extracted from *E. terschekii* have concomitant effects on larval viability in *D. buzzatii* but not of *D. koepferae* (Corio C., Soto I., Carreira V. and E. Hasson unpublished results).

Adult body size and starvation resistance are both positively correlated with the amount of reserve substances as lipid content in *D. melanogaster* (Harshman and Schmid 1998). In this context, it is interesting to note that, contrary to expectations, *D. buzzatii* has a smaller body size and greater starvation resistance than *D. koepferae*. Thus, we may hypothesize that different metabolic pathways may be differentially activated during larval development in flies growing in alternative resources. Thus, information on metabolic pools in flies reared in different cacti may be helpful to test this hypothesis. Pathways mainly involved in the metabolism of sugars and lipids may be differentially activated in *D. buzzatii*, whereas *D. koepferae* has probably evolved more efficient mechanisms of detoxification to develop in relatively more 'hostile' environments.

Finally, host selection by oviposition females offers a central theme in the study of insect-plant interactions. The basic setting for host plant choice, from an evolutionary standpoint, is that natural selection should favor females endowed with the ability to choose the most suitable host among several alternatives, particularly in cases in which larvae have limited mobility across rearing sites (reviewed in Craig and Itami 2008; Gripenberg et al. 2010). According to the preference-performance hypothesis females maximize fitness by choosing egg-laying sites where progeny performs best. On the one hand, our results indicate that only *D. buzzatii* seems to confirm the expectations of the preference-performance hypothesis since it performed better in its preferred natural host

for egg-laying. On the other hand, the case of *D. koepferae* is certainly puzzling, since flies neither prefer *E. terschekii* for egg-laying nor achieved the best indicators of performance in the cactus from which it emerges more frequently in nature. However, recent developments that take into consideration constraints of the feeding niche in the context of the preference-performance hypothesis may help to explain the general picture (Craig and Itami 2008; Gripenberg et al. 2010). It has been argued that habitat complexity and polyphagy may weaken the preference-performance relationship (Craig and Itami 2008; Gripenberg et al. 2010), as in the case of the generalists *D. melanogaster* and *D. simulans* (Soto et al. 2011). Also, this might be the case of *D. koepferae* since it has evolved the ability to use an ample array of columnar cacti of the genera *Cereus* and *Echinopsis* in Argentina (Hasson et al. 1992; Fanara et al. 1999) and *Neocardenasia* in Bolivia (Fontdevila et al. 1988) which may share the ability to produce different types of alkaloids, whereas *D. buzzatii* is mainly specialized on the relatively homogeneous habitat offered by prickly pears.

Future work aimed to identify genes involved in the differential use of alternative breeding resources may help to untangle the physiological mechanisms and the genetic basis of tolerance to alkaloids and, thus, the general mechanisms involved in host plant shifts.

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