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Pupal emergence pattern in cactophilic *Drosophila* and the effect of host plants

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Abstract *Drosophila buzzatii* and *D. koepferae* are sibling cactophilic species. The former breeds primarily on prickly pears (genus *Opuntia*) whereas the latter breeds on columnar cacti of the genera *Cereus* and *Trichocereus*, although with certain degree of niche overlapping. We examined the interspecific differences in diurnal temporal patterns of adult emergence from puparia and evaluated whether this behavior is affected by rearing in the different cactus hosts available in nature. We detected important host-dependent genetic variation for this trait differentially affecting the emergence schedule of these species. Diurnal pattern of emergence time was directly correlated with developmental time and negatively correlated with adult wing size, suggesting that early emergences are at least indirectly correlated with increased fitness. We discussed our results in terms of their putative effects on fitness and the genetic-metabolic pathways that would be presumably affected by host's nutritional-chemical differences.

Key words alkaloids; cactus; circadian cycle; life history traits; phenotypic plasticity; pupae

Introduction

Arthropods are a predominant animal taxon in arid environments (Whitford, 1999). Despite the extreme temperatures and low humidity that might constitute severe stressful conditions for their physiology, these invertebrates have managed to adapt and thrive in desert habitats. The tendency for nocturnal/crepuscular activity patterns may prevail in these environments, since it allows avoiding exposure to extremely hot conditions. In this regard, the daily activity patterns of desert flies are a good example of temporal avoidance as an adaptation to the thermal stress (Dahlgaard *et al.*, 2001). Some fly communities, including species of *Drosophila*, occur in arid environments (Markow & O'Grady, 2005; Oliveira *et al.*, 2012).

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However, desiccation and temperature stress can significantly impact on them, even in mesic environments (Worthen *et al.*, 1998; Worthen & Haney, 1999; Folguera *et al.*, 2008). As with other holometabolous insects, adaptive mechanisms in response to stress are likely to vary across life stages. Adults may display avoidance of extreme temperatures by seeking refuge in cooler/warmer microhabitats (Junge-Berberovic, 1996; Feder *et al.*, 2000), but larvae and pupae might be less able to spatially avoid thermal stress (Feder *et al.*, 1997) forcing them to adapt through metabolic adjustment (Hoffmann & Parsons, 1991; Dahlgaard *et al.*, 2001). Indeed, Coyne *et al.* (1983) have already suggested that the marked adaptive differences in thermal resistance observed in pupae (but not in adults) from different populations of *Drosophila*, are the result of the pupae's inability for behavioral response (i.e., lack of a mechanism for mobility) to extreme temperatures.

The timing of daily activity plays an important role in stress-avoidance adaptations as many aspects of

physiology and behavior are clock-controlled. There are numerous activities restricted to a particular part of the day or night in many insect species (reviewed in Saunders, 2002). For instance, mating presents 2 daily peaks of increased activity in natural populations of the cactophilic *D. mojavensis* Patterson (Krebs & Bean, 1991), one in the morning and one in the evening. Pupation and pupal emergence time display physiologic-related circadian rhythms as potential adaptations to avoid environmental stresses (Bartholomew, 1964; Saunders, 2002; Sørensen & Loeschcke, 2002). Although the phenomenon of insects emerging from the pupae following a diurnal rhythm was well established in the past century (e.g., Bliss, 1926; Pittendrigh, 1954; Bateman, 1955; Brett, 1955; Palmén, 1955; Bünning, 1958; Bakker & Nelissen, 1963), recent studies on the topic are rare or nonexistent for the vast majority of holometabolous groups.

The *Drosophila repleta* group has diversified in arid and semiarid regions of the Americas due to the ability of several species to colonize necrotic tissues of cacti (Throckmorton, 1975; Durando *et al.*, 2000). Cactophilic species are strongly associated with their host plants and show different degrees of specialization and niche width. Within this group, the *D. buzzatii* cluster constitutes a radiation occurring in the last 4.6 million years (Oliveira *et al.*, 2012). This monophyletic group of at least 7 species is mainly found in open xerophytic regions of South America (Manfrin & Sene, 2006). We studied the pair of sibling species *D. buzzatii* Patterson and Wheeler and *D. koepferae* Fontdevila and Wasserman, which co-occur in the arid Andean regions of western Argentina and Bolivia (Fontdevila *et al.*, 1988). *Drosophila buzzatii* uses necrotic cladodes of prickly pears of the genus *Opuntia* as primary hosts, whereas *D. koepferae* exploits mainly columnar cacti of the genera *Cereus* and *Trichocereus*. Nonetheless, in the vast areas where both species occur in sympatry, a certain degree of overlap in host exploitation occurs, with the 2 species emerging from both resources despite maintaining a preference for their respective primary host (Hasson *et al.*, 1992; Soto *et al.*, 2012). Previous studies showed that traits associated to fitness were maximized when flies were reared in their primary hosts (Soto *et al.*, 2008a,b, 2012, 2014; Hurtado *et al.*, 2012). Developmental instability levels increased when flies of both species were reared in their respective secondary host (Soto *et al.*, 2008b).

In contrast to abundant literature focused on morphological and life-history traits, studies addressing stress-avoidance traits and their ecological relevance are scarce for this group, despite the high amount of genetic variability known to be available to fuel the adaptive evolution of behavioral and physiological characters (Hoffmann, 1991; Boake, 1994; Soto *et al.*, 2012). In this regard, the best known behaviors are host acceptance and oviposition preference (Fanara *et al.*, 1999; Soto *et al.*, 2012). Host acceptance assays revealed that both types of cactus are equally accepted by *D. buzzatii* and *D. koepferae* as egg-laying sites. In contrast, oviposition preference assays revealed that *D. buzzatii* clearly prefers its primary host, whereas for *D. koepferae* the pattern is less clear as similar quantities of eggs were found to be laid in both hosts' tissues (Soto *et al.*, 2012).

In this study, we determined, described, and compared the daily patterns of adult emergence from puparia of *D. buzzatii* and *D. koepferae* in different cactus hosts. We evaluated whether the time of emergence of adults presents a plastic host-dependent pattern or is otherwise a canalized clock-controlled cycle (potentially related to exogenous predictable stress factor such as temperature). Additionally, we analyzed the existence of intraspecific genetic variation for this trait and its correlation with fitness-related traits.

Materials and methods

Fly stocks and cactus collection

Ten isofemale lines of each species were founded with flies collected in the Valle Fértil Natural Reserve, located in Northwestern Argentina (30°31′13″S, 67°34′05″W), where both *O. sulphurea* and *T. terscheckii* are available as cactus hosts. Rotting cladodes and fresh materials of both cacti were collected for the preparation of "seminatural" rearing media. The fresh cactus material was stored at –20 °C while the exudates from several necrotic tissues were maintained at 4 °C in plastic containers with sterilized cotton caps, with the addition of 10 g of fresh cactus every 2 weeks. For the preparation of the "seminatural" media, pieces of fresh cactus of each species were mixed in a blender and 6 mL were poured into standard glass vials. Vials were then inoculated with 0.1 mL of the fermenting juice produced by the necrotic tissues (see Fanara *et al.*, 1999 for details). Isofemale lines (lines hereafter) of each species were maintained in bottles with 30 mL of standard *Drosophila* instant medium (Carolina Biological Supply Company) until the onset of the experiments (2 generations after collection). Laboratory rearing and experimental conditions were held constant at 25 ± 1 °C with a 12 : 12 h light/dark photoperiod.

Collection permits for both flies and cacti tissues were issued to IMS and JP by local authorities of Conservation Management and Protected Areas (Secretariat of

Environment and Sustainable Development, San Juan, File No. 1300-0236-13).

Emergence time recording and traits scored

Drosophila buzzatii and *D. koepferae* larvae were reared in sets of vials prepared with *O. sulphurea* or *T. terscheckii* seminatural media. We set 2 egg-collecting chambers per species, containing a petri dish with egg-laying medium (agar–agar with commercial yeast) and 100 pairs of sexually mature flies. Petri dishes were removed 12 h later and inspected for the presence of eggs. The eggs were collected on the agar surface and then sterilized with 50 % clorox for 3 min (Fanara *et al.*, 1999); batches of 100 eggs were placed on sterile agar and incubated for another 24 h to allow larval hatching. Batches of 30 first instar larvae were then collected and placed in culture vials containing 6 mL of 1 of the 2 alternative seminatural cactus media (cactus hereafter). Larval placing was performed between 7 and 8 am. We analyzed 10 isofemale lines per *Drosophila* species, with 4 replicated vials initiated for each combination of line and cactus. Emerged flies were collected every 4 h (at 8, 12, 16, and 20 h) during the diurnal period (i.e., light hours in the photoperiod) and sexed under light CO_2 anesthesia.

Mean emergence time (ET) for each replicate was estimated by recording the number of emerged individuals in each collection interval, every day by a total of 5 d. Additionally, we scored 3 traits associated with the performance of genotypes in host exploitation: larval viability (LV), developmental time (DT) and adult wing size (WS). LV was scored for each vial as the proportion of adult survivors with respect to the number of larvae originally placed in the vial. DT was recorded as the time elapsed (in hours) from the moment in which first instar larvae were placed in the vials until the emergence of each adult fly. WS (as a proxy for body size) was scored following Soto *et al.* (2014) protocol. Briefly, right wings of adult flies of both sexes were removed and mounted on slides. Digital images of wings were obtained, 10 landmarks were placed along each wing and the centroid size (the square root of the sum of the squared distances of landmarks to the centroid of the configuration) was calculated.

Data analysis

The window of emergence from pupae encompassed 6 d although over 95% of all individuals usually emerged within the first 5 d. Thus, in order to avoid heavy tailed distributions, the individuals emerged on the sixth day were considered for LV analysis but treated as outliers and therefore excluded from the ET and DT analyses.

Regarding ET, as the response variable (number of emerged individuals) was measured as a count variable, the analyses were carried out using Generalized Linear Mixed Models (GLMM) with a Poisson distribution. To account for differences in viability, the total number of emerged individuals of the same sex from the same replica at the end of the experiment was used as covariable. Each *Drosophila* species was analyzed separately. Three fixed explanatory variables were considered: Cactus, Sex, and Hour of emergence (integer covariate representing each collection interval), as well as their interactions.

After data transformation, the traits complied with ANOVA assumptions. LV scores were angularly transformed whereas DT and WS scores were log-transformed. Traits were analyzed by means of an ANOVA with *Drosophila* and Cactus (fixed categorical factors, each one with 2 levels) and Line (categorical random factor with 10 levels, nested in *Drosophila*) as explanatory variables. Additionally, we performed intraspecific analyses consisting in two-way factorial ANOVAs with Cactus (fixed) and Line (random) as explanatory variables.

Correlations between ET and the other traits were tested for each line in both cacti by means of Pearson product moment correlation tests corrected for multiple tests. To assess the relative contributions of genetic and environmental sources of phenotypic variation, we included random intercept and slope models for Line and Replica (random factors). These designs are classic applications of the isofemale line technique for characterizing quantitative traits in natural populations (David *et al.*, 2005) in which the Line-associated variation is an estimator of phenotypic (genetic) variation, the Cactus-associated variation estimates the phenotypic plasticity and the interaction between them indicates a genetic by environment interaction (G × E). In ET analysis, we performed intraspecific Poisson GLMMs using the same fixed explanatory variables, as well as Line and Replica as random factors. Statistical significances for the latter were tested through likelihood ratio tests, by successively dropping fixed terms and assessing their contribution to the model.

Analyses were performed in R environment (R Core Team, 2015) using the packages lme4 (Bates *et al.*, 2014) and car (Fox & Weisberg, 2011).

Results

We recorded the emergence of 3085 total individuals (from which 26 adults emerged on the sixth day). Mean

Table 1 Means corresponding to emergence time (ET, mean hour of daily emergence from puparia), larval viability (LV, estimated as the proportion of original larvae emerged as adults), developmental time (DT, in hours), and wing size (WS, log centroid size) of isofemale lines of *D. buzzatii* and *D. koepferae* reared in alternative cacti. Standard errors are reported in parentheses.

Drosophila species	Cactus	ET	LV	DT	WS
D. buzzatii	*O. sulphurea*	11.63 (0.23)	0.75 (0.03)	296.24 (2.29)	39.56 (0.3)
	T. terscheckii	11.49 (0.24)	0.59 (0.03)	272.15 (1.75)	39.55 (0.3)
D. koepferae	*O. sulphurea*	10.88 (0.17)	0.61 (0.03)	279.25 (1.87)	39.74 (0.07)
	T. terscheckii	10.30 (0.18)	0.62 (0.03)	275.00 (2.37)	39.71 (0.07)

values of ET, LV, DT, and WS for each breeding cacti are shown in Table 1.

Emergence time

Sexual differences in ET were absent in both species (Table 2). In the case of *D. buzzatti*, the proportion of emerged adults peaked late in the morning (collected at 12 h), whereas adults of *D. koepferae* revealed high proportions of emerged flies both at 8 and 12 h (Fig. 1). The interaction between Hour of emergence and Cactus was significant only in the case of *D. buzzatti*, revealing that the pattern of emergence differed between rearing hosts with individuals emerging earlier during the day when they are reared in *T. terscheckii* (Table 2). Genetic variation was detected in both species but was higher in *D. koepferae* (Line variance $_{D. buzzatti}$ = 0.006; Line variance $_{D. koepferae}$ = 0.015). Moreover, the Replicate factor (a proxy of environmental "noise") explained a larger amount of total variation than the Line factor in both species, but was higher and only significant in *D. koepferae* (Replicate variance $_{D. buzzatti}$ = 0.084; Replicate variance $_{D. koepferae}$ = 0.313). Thus, on average, random

environmental variation was 18 times larger than genetic induced variability.

Performance of genotypes in host exploitation: larval viability, developmental time, and adult wing size

The analysis of LV showed a *Drosophila* by Cactus significant interaction (Table 3). *Drosophila buzzatii* presented lower LV when reared in *T. terscheckii* (*post hoc* Tukey's test, $F_{1,120}$ = 15.513, P < 0.01) whereas no differences were detected for *D. koepferae* (*post hoc* Tukey's test, $F_{1,120}$ = 0.001, P = 0.983) (Fig. 2). Additionally, important variation among lines within *Drosophila* species was also detected (Fig. 2). Thereby no significant Cactus by Line interaction was observed in general or intraspecific analyses suggesting the nonexistence of variation of reaction norms among genotypes (Table 3). Regarding the variance components, we observed extremely differing patterns between species. Although neither species showed significant G × E interaction, *D. buzzatii* displayed low levels of phenotypic variation attributable to among-genotypes variation, whereas variation among

Table 2 Intraspecific analyses of deviance of emergence time (Proportion of emerged adults).

Source of variation	*D. buzzatii*			*D. koepferae*		
	χ^2 value	df	*P* value	χ^2 value	df	*P* value
Hour	37.397	1	9.7^{-10}***	48.586	1	3.2^{-9}***
Cactus	3.363	1	0.067	0.289	1	0.59
Sex	1.219	1	0.269	1.172	1	0.278
Cactus × hour	3.864	1	0.048*	0.258	1	0.612
Sex × hour	0.842	1	0.359	0.034	1	0.852
Cactus × sex	0.235	1	0.628	0.292	1	0.589
Cactus × sex by hour	0.025	1	0.875	1.014	1	0.314

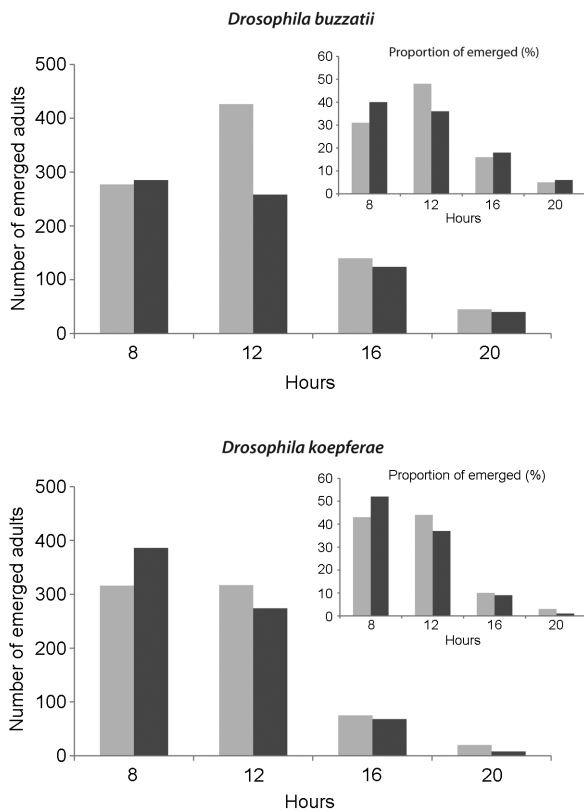df: degrees of freedom; *P < 0.05; ***P < 0.001.

Fig. 1 Emergence time. Total number (and proportion from the total) of adults emerged in each cactus host in the 4-h periods of diurnal collection (light gray: *O. sulphurea*; dark gray: *T. terscheckii*).

lines accounted for 38% of the overall LV variation in *D. koepferae* (Fig. 2).

On average, both species developed faster (lower DT) in *T. terscheckii* but as for LV, the difference was more marked in *D. buzzati* (Fig. 2). This behavior was not entirely consistent among lines within species, indicating substantial amounts of host-dependent genetic variation segregating in the populations (significant Cactus by Line interaction, Table 3). Host cacti influence on *D. buzzatii*'s DT, but also an important G × E interaction was detected indicating significant amounts of variation in phenotypic plasticity (39% of total phenotypic variation; Fig. 2). *Drosophila koepferae*'s mean DT on the other hand, seemed rather impervious to changes in rearing host. Nevertheless, an important variation among the genotypes was observed for this trait, with the Line factor accounting for 19% of total phenotypic variation (Fig. 2).

Regarding WS, *D. koepferae* presented bigger wings than its sibling. In a pattern opposite to that of DT, *D. buzzatii* displayed size variation among lines but not host-dependent phenotypic plasticity whereas for *D. koepferae*

there was an evident variability among genotypes across hosts (Table 3, Fig. 2).

Correlations among traits

Both *Drosophila* species presented mean Emergence times significantly correlated with developmental time and wing size of adults but not with larval viability (Table 4). The daily-belated genotypes of both species displayed longer developmental times throughout the experiment (a seemingly trivial result but that discards the scenario of flies emerging mainly in the afternoon of the first day of emergence and in the morning of subsequent days). Also, both species showed smaller wing sizes as adults. Finally, whereas *D. buzzatii* had similar pattern of correlations between traits independently of the cactus, *D. koepferae* presented a host-dependent pattern of correlations (Table 4).

Discussion

The temporal pattern of emergence is regarded as an important life history trait in holometabolous insects inhabiting regions where, such as deserts, environmental conditions are highly variable reaching potentially stressful levels with lethal values for many organisms (Hoffmann & Parsons, 1991; Feder *et al.*, 1997; Dahlgaard *et al.*, 2001). In the case of flies, after emergence from puparia, adults are vulnerable and unable to immediately take off. Flies expend a couple of hours extending their wings and hardening their cuticle before being able to fly. During this critical period, they have limited mobility and are under potentially higher risk of predation and or desiccation. Therefore, the emergence schedule should be an adaptive trait highly linked with local environmental conditions. In this study, we have established that emergence events are not randomly distributed during the day as both species exhibit an early daytime emergence. In natural (i.e., collection site) conditions, this is when milder and moister environmental conditions prevail (AccuWeather, 2015). Our results are in agreement with previous observations in *D. pseudoobscura* Frolova which emerges from the puparia close to dawn when the relative humidity of the air is at its height. In fact it has been established that the maximum eclosion's success in this species occurs during dawn (Pittendrigh, 1958). Bakker and Nelissen (1963) found that for *D. melanogaster* Meigen, emergence is highest between 4 and 8 o'clock in the morning. However, the eclosion's success along humidity and temperature gradients in cactophilic species remains undescribed.

Table 3 Inter and intraspecific mixed ANOVAs for larval viability, developmental time, and wing size.

Source of variation	Effect	Larval viability			Developmental time			Wing size		
		df	MS	*F*	df	MS	*F*	df	MS	*F*
Both *Drosophila* species										
Drosophila	Fixed	1	0.141	1.72	1	2001	5.82[*]	1	1136	6.40[*]
Cactus	Fixed	1	0.234	9.49[**]	1	8028	30.27[***]	1	0.014	4.08
Drosophila × Cactus	Fixed	1	0.259	10.46[**]	1	3937	14.85[**]	1	0.01	0.35
Line (*Drosophila*)	Random	18	0.082	3.31[**]	18	343	1.29	18	0.178	51.19[***]
Cactus × Line (*Drosophila*)	Random	18	0.025	1.27	18	265	1.95[*]	18	0.003	2.35[**]
Error		120	0.019		120	136		120	0.001	
D. buzzatii										
Cactus	Fixed	1	0.493	20.86[**]	1	11605	28.68[***]	1	0.004	3.08
Line	Random	9	0.043	1.81	9	267	0.66	9	0.351	307.17[***]
Cactus × Line	Random	9	0.024	1.1	9	405	3.50[**]	9	0.001	0.54
Error		60	0.021		60	115		60	0.002	
D. koepferae										
Cactus	Fixed	1	0.001	0.01	1	360	2.87	1	0.012	2.04
Line	Random	9	0.121	4.67[*]	9	419	3.34[*]	9	0.005	0.80
Cactus × Line	Random	9	0.026	1.46	9	126	0.8	9	0.006	6.92[***]
Error		60	0.018		60	157		60	0.001	

df: degrees of freedom; MS: mean squares. [*]$P < 0.05$; [**]$P < 0.01$; [***]$P < 0.001$.

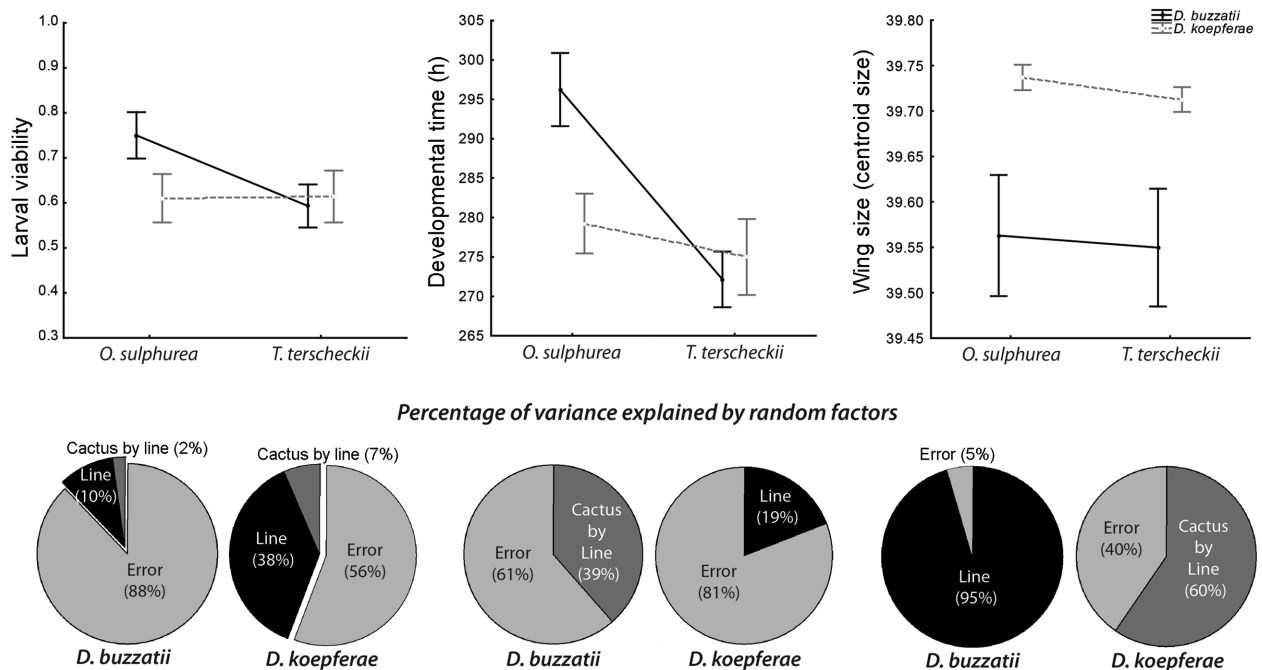*Percentage of variance explained by random factors*

Fig. 2 Larval viability, developmental time and wing size displayed by each *Drosophila* species reared in both cacti hosts. In the bottom, the percentage of variance explained by random sources of variation is shown for each life history trait.

Table 4 Correlation patterns. Pearson correlation coefficients among emergence time (ET) and fitness-related traits: larval viability (LV), developmental time (DT), and wing size (WS). Values correspond to rearing in both hosts: *Opuntia sulphurea* (above diagonal) and *Trichocereus terscheckii* (below diagonal). Asterisks denote significant correlations ($P < 0.05$).

	D. koepferae				*D. buzzatii*			
	LV	DT	WS	ET	LV	DT	WS	ET
LV	–	0.355[*]	−0.219[*]	0.158	–	0.376[*]	−0.157	0.085
DT	0.141	–	−0.345[*]	0.524[*]	0.351[*]	–	−0.093	0.291[*]
WS	−0.044	0.109	–	−0.303[*]	−0.162	−0.131	–	−0.284[*]
ET	0.107	0.339[*]	−0.285[*]	–	0.136	0.411[*]	−0.330[*]	–

The number of individuals emerging during the night was negligible, as the flies first scored in the morning were still in their puparia at 7 am when the record began (IM Soto, personal observation) and according to our capture records at the collection site, *D. buzzatii* and *D. koepferae* are more active in the 2–3 h following sunrise and the hours preceding dusk. In this regard, the next step is to confirm these observations in both natural conditions and experimental setups, and extend them to other related species inhabiting xeric environments.

Studies of natural populations in the field and under laboratory conditions have shown that *D. buzzatii* and *D. koepferae* differ in their preferences regarding oviposition sites (Soto *et al.*, 2012) and higher viabilities, faster developing times and increased mating success for both species were observed when they were reared in their respective primary hosts (Soto *et al.*, 2008a; Hurtado *et al.*, 2012). Here we enlarged the list of traits in which host-dependent phenotypic plasticity is displayed by *D. buzzatii* whereas *D. koepferae* showed a more canalized behavior as previously observed for viability, developmental time (Soto *et al.*, 2008a,b, 2012, 2014), starvation resistance, oviposition preference (Soto *et al.*, 2012), and genital morphology (Soto *et al.*, 2007). As a performance-related trait, time of pupal emergence was positively correlated with developmental time and negatively correlated with adult wing size. Previous results, in *D. melanogaster*, have shown that food uptake affects puparium formation and emergence time (Bakker, 1959). Thus, for cactophilic species, variation in larval density (as in field rots where densities may be much higher) might produce results different than those observed in the present study. Larval density used in our experiments (30 larvae per vial) has proven to be optimal for different fitness-related traits (i.e., it does not change results obtained using lower larval densities; Fanara *et al.*, 1999) and yet was sufficient to elicit a host-dependent response. More stressful conditions and the

assessment of a reaction norm to emergence time remain untested.

Similar correlations between emergence time and developmental time and adult size have been observed in *D. melanogaster* (Bakker & Nelissen, 1963). Delayed development and small adult size are considered indicators of low performances (Norry *et al.*, 2001; Cortese *et al.*, 2002). These correlations would indicate that, within hosts, early emergences are at least indirectly, if not directly, correlated with increased fitness. Regarding the developmental time-wing size correlation, our results showed that this negative correlation, expected in *Drosophila* species in general (Bakker & Nelissen, 1963; Norry *et al.*, 2001; Cortese *et al.*, 2002), was not observed in most cases except for *D. koepferae* bred in *O. sulphurea*. It is interesting to note that the patterns of correlation between traits in *D. koepferae* changed with breeding resources, unlike *D. buzzatii*. This may affect the evolution of different strategies of adaptation to heterogeneous breeding resources.

D. buzzatii showed and interesting variation in the time of emergence from pupae between cactus hosts while *D. koepferae* tended to be more resilient (a pattern previously observed on other traits; Soto *et al.*, 2007; Soto *et al.*, 2008b; Soto *et al.*, 2014). Previous observations could be considered in the context of the present results. First, it is known that the nutritional quality of food has profound effects on the circadian rhythms of many organisms (Panda *et al.*, 2002; Froy, 2009). In fact, in *Drosophila*, correlations between the circadian clock and development time have been observed, with the latter depending in turn on the substrate quality (Walkiewicz & Stern, 2009; Yadav & Sharma, 2013). In the present case, *O. sulphurea* is a cactus host richer in sugar and fat content respect to *T. terscheckii* (Padro & Soto, 2013; Carreira *et al.*, 2014), which also harbors higher diversity of cactophilic microorganisms and yeasts in its necrotic tissues (Mongiardino Koch *et al.*, 2015). Therefore, the experimental substrates are nutritionally different

and could be a key factor conditioning the time spent in pupal stage, thus affecting ET as we know it affects development time (Soto *et al.*, 2008b; Soto *et al.*, 2014).

Cactus hosts also display extremely different contents of secondary metabolites (terpenes and alkaloids). There is substantial empirical evidence in insects showing pronounced modifications of behavioral, morphological, biochemical, and physiological traits in response to secondary plant compounds (Fogleman, 2000; Narberhaus *et al.*, 2005; Padró *et al.*, 2014; Agrawal & Weber, 2015). In particular, alkaloids have a wide spectrum of pharmacological effects, including the alteration of circadian rhythms (Onishi *et al.*, 2012a,b). Psychotropic drugs have been observed to affect the circadian rhythm of locomotor activity in insects, which along with the mescaline serotonin binding receptor emphasize the possible implication of neurotransmitters pathways affecting the emergence time via the central nervous system (Cymborowski & Muszyńska, 1974; Cymborowski, 1998; Aghajanian & Marek, 1999; Nishinokubi & Tomioka, 2000; Yuan *et al.*, 2005). On the other hand, the presence of high concentrations of alkaloids compromise metabolism via detoxification pathways. In this sense, *T. terscheckii* is naturally rich in mescaline and close-related to phenylethylamine alkaloids with psychotropic properties (Reti & Castrillon, 1951; Corio *et al.*, 2013). Previous studies showed that *D. buzzatii* but not *D. koepferae* suffered the effects of increasing doses of *Trichocereus*' alkaloids (Soto *et al.*, 2014).

Therefore, direct and indirect mechanisms modulating the circadian clock depending on the alkaloid-molecular target could be involved in the present case. For instance, in *Drosophila*, it has been widely established the implication of the cytochrome P-450 inducible enzymes family in the metabolism of cactus alkaloids, whose activity in turn can affect circadian rhythms (Danielson *et al.*, 1995; Matzkin *et al.*, 2006; Bono *et al.*, 2008; Froy, 2009; Pan *et al.*, 2009). Recently, it has been proved that the same enzymes are differentially expressed in *D. buzzatii* and *D. koepferae* when exposed to alkaloids (De Panis *et al.*, 2016). In this sense, the genomic expression profile in cactophilic *Drosophila* has been observed to be largely plastic during development responding to different cactus hosts, causing pervasive changes beyond the expected specific chemical–stress responses and comprising genes directly related to circadian cycles (Matzkin *et al.*, 2006; Etges *et al.*, 2015; De Panis *et al.*, 2016).

In summary, host plant differentially affect daily emergence patterns in the studied species, which nonetheless showed a strong fixed pattern of early eclosion during the day. The fact that this behavior correlates with the moister/cooler environmental conditions of the day suggest the overwhelming relevance of physical conditions during emergence time adaptation. Further field studies in natural populations should be performed in order to test these hypotheses and to assess the ecological implications of different emergence strategies.

Acknowledgments

Disclosure

The authors declare no conflict of interest.

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