The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2013, **109**, 342–353. With 5 figures

An alkaloid fraction extracted from the cactus *Trichocereus terscheckii* affects fitness in the cactophilic fly *Drosophila buzzatii* (Diptera: Drosophilidae)

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Received 29 November 2012; revised 3 January 2013; accepted for publication 3 January 2013

The host-plant environment of phytophagous insects directly affects various aspects of an insect's life cycle. Interestingly, relatively few insect groups have specialized in the exploitation of plants in the Cactaceae family, potentially because of the chemical and ecological challenges imposed by these plants. The cactophilic *Drosophila buzzatii* Patterson & Wheeler, 1942 is a well-studied model in evolutionary ecology, partially because of its ability to exploit toxic cactus hosts. Previous studies have shown a negative effect on performance when flies are reared in an alternative columnar cactus host of the genus *Trichocereus*, relative to its primary cactus host, *Opuntia*. These observations were attributed to the presence of alkaloids in *Trichocereus* tissues, a chemical deterrent to herbivores that indirectly affects *Drosophila* larvae; however, the putative toxic effect of alkaloids has never been tested directly in *D. buzzatii*. The present study is the first attempt to relate chemical extracts in *Trichocereus terscheckii* Britton & Rose, 1920 with detrimental effects on *D. buzzatii*. We assessed the effects of a crude alkaloid extract, rich in phenylethylamines, and a 'non-alkaloid fraction' on viability and adult wing morphology. Our results indicate that rearing larvae on an artificial diet containing different concentrations of the crude alkaloid extract decreased pupal viability and adult size in a concentration-dependent manner. We discuss the role of cactus alkaloids in the evolution of host-plant use in cactophilic flies.

ADDITIONAL KEYWORDS: alkaloids – cactus – host shifts – viability – wing morphology.

INTRODUCTION

Host plants represent the primary environment for phytophagous insects, and, as such, affect various aspects of an insect's life cycle (Schoonhoven, van Loon & Dicke, 2006). Plants have evolved a variety of defences to avoid insect attacks, including chemical defences that impose serious challenges to larvae developing in close contact with the host, and/or to the adults that use the plant as a shelter microhabitat or as food (Kircher, 1982; Schoonhoven *et al*., 2006).

Insect groups specialized in the exploitation of Cactaceae face a variety of chemical and ecological challenges (Nobel, 2002). The radiation of Cactaceae has been accompanied by the evolution of a broad array of secondary metabolites (allelochemicals). For example, typical allelochemicals of some columnar cacti include terpenoids that serve as feeding deterrents and isoquinoline alkaloids that obstruct neurotransmission (reviewed in Fogleman & Danielson, 2001). Nevertheless, cactus necroses are hosts to large arthropod communities (e.g. Castrezana & Markow, 2001).

The genus *Drosophila* comprises an impressive number of species groups of saprophytophagous flies that breed on the necrotic tissues of a wide variety of plant taxa, as well as feed on the microorganisms

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associated with the decomposition process (Markow & O'Grady, 2008). The Neotropical *Drosophila repleta* group includes several species that invaded the American deserts because of the acquisition of the ability to use decaying cacti as food and breeding substrates (Throckmorton, 1982; Wasserman, 1982). Cactophilic *Drosophila* have long constituted excellent models to investigate adaptation to a relatively narrow spectrum of potential host cacti. The classical studies of the cactus–yeast–*Drosophila* model system in the Sonoran Desert of south-western USA and north-western Mexico, is perhaps the best example (Fogleman & Danielson, 2001). Years of research in this model system led to two main conclusions: (1) fly species independently evolved unique sets of adaptations to live in deserts; and (2) the chemistry (presence of toxic compounds and nutritional sufficiency) of the cactus hosts is one of the major determinants of host-plant specificity (Fogleman & Abril, 1990; Fogleman & Danielson, 2001).

The South American cactophilic *Drosophila buzzatii* Patterson & Wheeler, 1942 belongs to the *D. buzzatii* cluster, an ensemble of at least seven species in different stages of divergence and varying degrees of host specialization (Manfrin & Sene, 2006; Hasson *et al*., 2009). *Drosophila buzzatii* has a remarkable preference for laying eggs on the decaying cladodes of *Opuntia* cacti (prickly pears; Soto *et al*., 2012), but also emerges from necrotic tissues of columnar cacti of the genera *Cereus* and *Echinopsis* (Hasson, Naveira & Fontdevila, 1992). However, flies reared in columnar cacti exhibit decreased survival, smaller body size, reduced starvation resistance, and extended developmental time relative to flies reared in *Opuntia* cacti (Fanara, Fontdevila & Hasson, 1999; Carreira *et al*., 2006; Fanara *et al*., 2006; Hasson *et al*., 2009; Soto *et al*., 2012). These observations have been interpreted as adaptations for exploitation of *Opuntia* cacti that differ markedly from columnar cacti in their temporal and spatial predictability (Fanara *et al*., 1999; Carreira *et al*., 2006; Soto *et al*., 2012).

The chemical properties of host cacti are considered to be one of the most important ecological differences between cactus species. Unlike *Opuntia*, columnar cacti produce toxic compounds such as alkaloids, medium-chain fatty acids, sterol diols, and triterpene glycosides. The alkaloids are considered to be the primary factor explaining the patterns of host-plant use in cactophilic *Drosophila* from the Sonoran Desert (Fogleman & Heed, 1989). Some of these compounds have been shown to affect mating behaviour, larval viability, rate of development, and adult longevity and fecundity (Heed & Mangan, 1986; Etges, Veenstra & Jackson, 2006). These chemical particularities of the Cactaceae have led to the suggestion that each cactus host may represent a different chemical environment for the larvae developing in the decaying plant tissues and the adult flies feeding on the same substrate (Hasson *et al*., 1992; Fanara *et al*., 1999; Fernández Iriarte & Hasson, 2000); however, whether differences in cactus chemistry are the primary factors shaping patterns of host plant use in *D. buzzatii* is an open question.

Little is known about the chemistry of *Trichocereus terscheckii* Britton & Rose (1920), and even less is known about the chemical constituents responsible for the detrimental effects observed in *D. buzzatii* reared on *T. terscheckii* (reviewed in Hasson *et al*., 2009).

In the present study, we assess the effects of a crude alkaloid fraction and a non-alkaloid fraction extracted from *T. terscheckii* on viability and wing morphology (both size and shape) as indicators of performance of *D. buzzatii*. Our long-term goal is to elucidate the role of cactus chemistry in the evolution of host-plant use in the *D. buzzatii* cluster.

MATERIAL AND METHODS

Drosophila stocks

Flies were collected by net sweeping on fermented banana baits in a site close to San Agustín del Valle Fértil (30.3°S, 67.3°W, San Juan Province, Argentina) in March 2008. In the collecting area, *D. buzzatii* breeds and feeds primarily on the rotting cladodes of *Opuntia sulphurea*, and secondarily on the decaying stems of *T. terscheckii*. Flies were sexed upon arrival in the laboratory and were then used to generate isofemale lines (lines hereafter) by placing individual females in vials containing 5 mL of Instant *Drosophila* Medium (Carolina Biological Supplies).

An outbred stock was generated by mixing equal numbers of flies of the progeny of the fourth laboratory-bred generation of 30 isofemale lines. This fly stock was housed under controlled laboratory conditions (25 ± 1 °C; mean relative humidity, 60 ± 10%; 12-h photoperiod, LD 12) until the experiments began in July 2008.

Extraction of plant chemicals

Samples of aerial parts (stem) of fresh *T. terscheckii* were collected in San Juan Province (north-western Argentina), at the same collection site as the flies. Stems were cut in ~0.5-kg slices and ground in a blender, mixed with EtOH 96% (580 g tissue in 0.6 L), and then filtered to remove solid material. The extractions were carried out by partitioning the concentrated EtOH with diluted acid as described in Ogunbodede *et al*. (2010), with minor modifications. The extract was evaporated to an aqueous suspension

at 40 °C in a rotatory evaporator and acidified with 500 mL of 10% HCl. The aqueous acidic fraction was partitioned between CH_2Cl_2 (extracted three times with 500 mL) and water to yield a dichloromethane fraction and a water-soluble fraction. The former fraction was evaporated in a rotatory evaporator yielding a non-basic fraction of 429.2 mg containing acid liposoluble compounds (e.g. terpenoids, fatty acids, sterols, and aromatic and other compounds). This fraction (hereafter referred to as the 'non-alkaloid fraction') was included as a separate treatment in the experiments described below to investigate its possible biological effects, as we did not know which fraction contained potential toxic compounds (other than alkaloids) responsible for the detrimental effects observed in *D. buzzatii*. The aqueous acidic fraction yielded a crude total 'alkaloid fraction' of 191.4 mg. Both the crude alkaloid fraction and the non-alkaloid fraction were solubilized in dimethyl sulfoxide (DMSO 100 μg mL^{-1}) and incorporated into the artificial diets used in the bioassays (Nair, Aremu & van Staden, 2011).

Identification of main basic compounds

Identification of the major compounds was accomplished using a coupled gas chromatography (GC)–mass spectrometer (MS) (Thermo Scientific EM/DSQ II–Trace GC Ultra AI3000) using a capillary GC column (30 m × 0.25 mm Rxi-5ms) fitted with an 'on-column' injector coupled to the MS. The conditions were as follows: injector 250 °C; temperature programme 70 °C (1 min), increasing by 10 °C min^{-1} to 290 °C (10 min); splitless mode (2 min), and carrier gas He (108 935.5 Pa; 1.5 mL min^{-1}). This methodology has proven to be highly efficient in the extraction of alkaloids from plant tissues, except for some natural alkaloid quaternary ammonium salts (Gan *et al.*, 2010).

Experimental treatments

To obtain experimental flies, 100 pairs of sexually mature flies of the outbred stock were released in egg-collecting chambers with a Petri dish containing an egg-laying medium (agar 2% + 5 mL of 3 parts ethanol : 1 part 60% acetic acid). Petri dishes were removed after 12 h, inspected for the presence of eggs, and incubated for another 24 h to allow for egg hatching.

As we estimated the quantity of 'alkaloids' per gram of fresh (or dried) tissue of cactus, we were able to control its concentration in the vials. The alkaloid extraction yielded 191.4 mg from a total of 580 g of fresh tissue (or 2.61 g of dry tissue). This suggests that the alkaloid concentration in *T. terscheckii* fresh tissue is about 0.33 mg per gram of wet weight (or 4.50 mg per gram of dry weight, 0.3%). These results are concordant with studies showing that *T. terscheckii* may contain 0.25–1.2% alkaloids (Reti & Castrillón, 1951).

Batches of 30 first-instar larvae were transferred to culture vials containing 8 g of instant laboratory medium hydrated with the same volume (4 mL) of: (1) water plus the same quantity of DMSO used to solubilize the alkaloid and non-alkaloid fractions (control vials); or (2) water solutions containing three different quantities of the alkaloid (A treatment) or the non-alkaloid fractions (NA treatment) previously solubilized in DMSO (see above). Ten replicated vials were run for each of six treatments plus the control: three testing the effect of increasing doses of the alkaloid extract (1× 'A1', 1.5× 'A2', and 2× 'A3' parts of the alkaloid fraction), and the remaining three testing the biological effect of increasing doses of the non-alkaloid fraction (1× 'NA1', 1.5× 'NA2', and 2× 'NA3' parts). Thirty first-instar larvae were transferred to each culture vial. Thus, a total of 2100 larvae (300 larvae per treatment) were seeded in vials for this study. The alkaloid concentration in A1 vials may be considered similar to the native concentration in the plant; however, as alkaloids in naturally rotting stems may reach higher concentrations as a result of the evaporation of water (Meyer & Fogleman, 1987), we also tested 1.5× and 2× concentrations of both fractions. These concentrations simulate variation that may occur during the desiccation of cactus tissues in nature, and allowed us to explore the biological effects of the respective fractions. Vials were incubated at 25 ± 1 °C with a 12–h light/12-h dark photoperiod until the emergence of adults.

Fitness-related traits

We estimated larval viability as the proportion of larvae seeded that reached the pupal stage in each vial (e.g. the number of pupae in each vial per 30 larvae seeded), and similarly pupal viability as the proportion of pupae that successfully completed metamorphosis and reached the adult stage in each vial (e.g. the number of emerged adults per the number of pupae). Combined values of larval and pupal viability in each vial provide an estimate of first-instar larva to adult viability, which is a major component of early fitness and performance (i.e. number of adults per 30 seeded larvae).

Emerged adults were collected, sexed, and the right wing of each fly removed and mounted on a slide. Images of wings were captured using a digital camera mounted on a binocular microscope (10×). Ten landmarks were digitized using TpsDig (Rohlf, 2003) at vein intersections (Fig. 1).

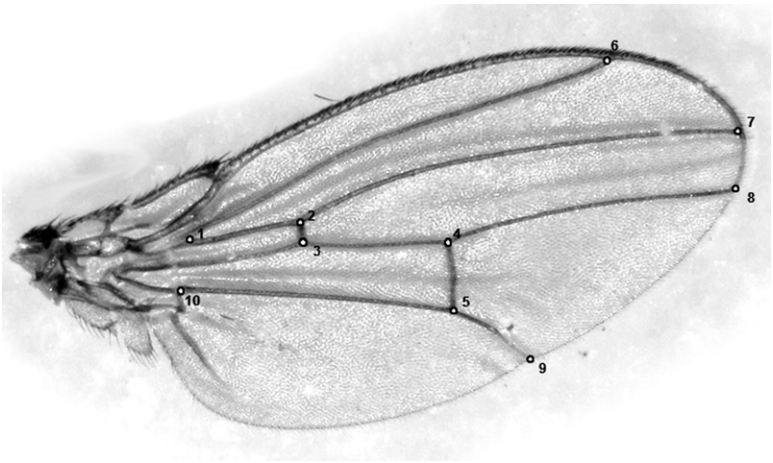

Figure 1. Dorsal view of the right wing and landmark positioning for morphometric analyses.

In *D. buzzatii*, body size is known to be related to mating success, longevity, avoidance of predation, fecundity, and tolerance to heat, cold, and starvation, among other traits (Cortese *et al*., 2002, and references therein). Wing length is a good proxy of body size known to be involved in a trade-off with developmental time in *D. buzzatii* (Fanara *et al*., 1999; Fernández Iriarte & Hasson, 2000; Cortese *et al*., 2002). Wing morphology was analysed by separating size from shape variation using geometric morphometric techniques (Bookstein, 1996). As a measure of wing size, we calculated the centroid size of each individual configuration of landmarks (the square root of the sum of the squared distances of each landmark to the centroid of the configuration in arbitrary units; Dryden & Mardia, 1998). Wing shape variation was investigated using the Procrustes technique. Shape coordinates were computed using a least-squares Procrustes superimposition method, where all wings are superimposed for the examination of differences in the position of landmarks once original variations in size, position, and orientation of the wings have been eliminated (Bookstein, 1996; Dryden & Mardia, 1998). This procedure created new shape variables (Procrustes coordinates) and eliminated four degrees of freedom, resulting in 16 shape–space dimensions (see Klingenberg, McIntyre & Zaklan, 1998). Relative warps analysis, a principal component analysis (PCA) of the matrix of shape scores, was performed with those coordinates to investigate the main trends in shape change. Thus, 16 new variables describing wing shape (relative warps) were generated. These variables consisted of scores corresponding to partial contributions of hierarchically scaled vectors spanning a linear shape space (Bookstein, 1991; Rohlf & Marcus, 1993). Relative Warp Analysis was performed using tpsRelw (Rohlf, 2003).

Statistical analyses

Viability differences among treatments were tested by means of one-way ANOVAs with 'treatment' as the main fixed factor. Viability data were angularly transformed (arcsin of the square root of the proportion) prior to the ANOVAs.

Wing-size differences among treatments were tested by means of a two-way ANOVA with 'treatment' and 'sex' as main fixed factors. Prior to statistical analyses, wing-size data were log transformed to meet the assumptions of the ANOVA.

Wing-shape differences among treatments were tested by means of a two-way MANOVA with 'treatment' and 'sex' as the main fixed factors, using the relative warp scores matrix as dependent shape variables.

To identify which treatments were responsible for the significant results in the ANOVAs for viability and wing size, we employed Dunnett tests that consist of pairwise comparisons of means between each treatment and the control (Zar, 1996).

Additionally, we performed linear regression analyses of total viability and female wing size (males could not be analysed because very few males survived in the most extreme alkaloid, A3, treatment) on alkaloid or non-alkaloid concentration as independent variables.

All statistical analyses were performed using general linear models implemented in STATISTICA 6.0 (Statsoft, 2001).

RESULTS

VIABILITY

Mean viabilities in each treatment are reported in Table S1. The ANOVAs for larval viability and total viability revealed significant differences among treatments (Table 1). Total viability in A2 and A3 treatments was lower than in the control ($P = 0.025$ and $P < 0.001$, respectively; Fig. 2a). In the non-alkaloid treatments, total viability in NA3 was higher than in the control ($P = 0.043$; Fig. 2b). In contrast to total viability, larval viability in vials exposed to alkaloid treatments did not differ significantly from the control ($P > 0.05$ in all cases), suggesting that mortality differences between treatments mainly occurred during the pupal stage (Fig. 2).

Regression analyses showed that total viability decreased as alkaloid concentration increased ($N = 30$, slope = –0.29, $R^2 = 0.55$, $P < 0.001$), whereas increasing proportions of the non-alkaloid fraction did not affect total viability ($N = 30$, slope ≈ 0, $R^2 < 0.003$, $P > 0.5$). Control vials were not included in the regression analyses.

Table 1. Results of ANOVAs testing for differences among treatments for pupal and total (larvae to adult) viabilities: degrees of freedom (d.f.), mean squares (MS) and statistic value (F)

Trait	Sources of variation	d.f.	MS	F
Larval viability	Treatment	6	0.11	5.34**
	Error	63	0.02	
Total viability	Treatment	6	0.37	29.23**
	Error	63	0.01	

**$P < 0.001$.

WING MORPHOLOGY

All flies with undamaged and normal wing venation patterns were included in the analysis of wing morphology. In general, large numbers of flies exposed to alkaloid treatments were excluded from the analysis because of a failure in wing unfolding or because of abnormal wing venation patterns that affected a vein usually employed in landmark positioning (60% in A2 and more than 90% in A3 treatments). In fact, only a few females emerged in A3 vials, and very few males and females exposed to the A2 treatment could be included in the analyses. The mean values of wing size of flies exposed to different concentrations of the alkaloid and non-alkaloid fractions are given in Table S2. The results of the ANOVA and the MANOVA revealed significant differences among treatments for wing size and wing shape, respectively (Table 2). Moreover, the treatment–sex interaction was significant for both aspects of wing morphology, suggesting that flies exposed to the alkaloid extract differed in the degree of sexual dimorphism, or, in other words, that differences between sexes depended on the presence/absence of the alkaloid extract. This effect was particularly strong in the wing size of flies exposed to the alkaloid extract (Fig. 3a). Pairwise comparisons using Dunnet's method showed that

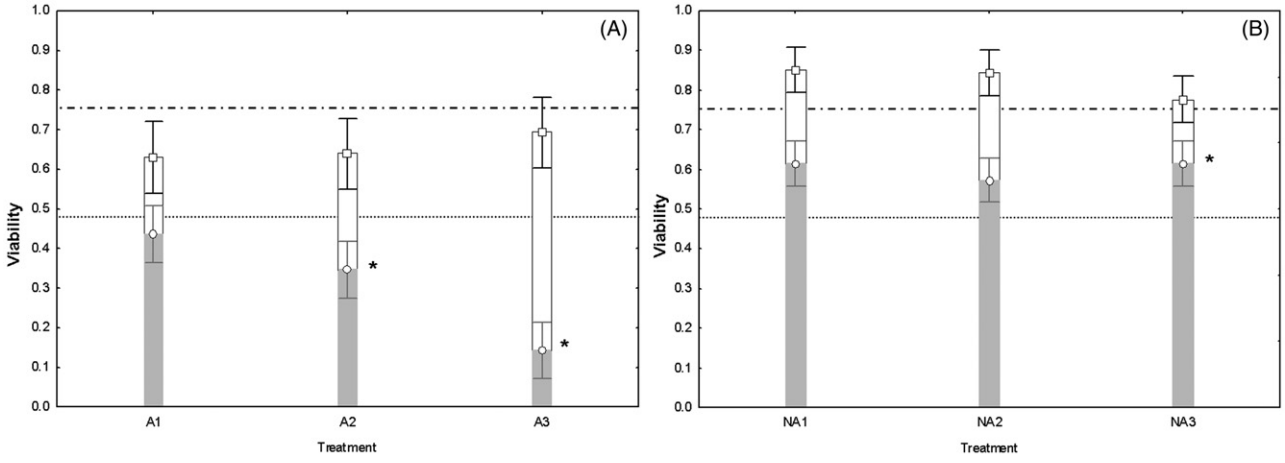

Figure 2. Larval (white bars) and total viability (grey bars) in *Drosophila buzzatii* reared in: (A), standard laboratory medium plus increasing concentrations of the alkaloid fraction of *Trichocereus terscheckii* (A1, A2, and A3, see text for details); and (B) standard laboratory medium plus increasing concentrations of the non-alkaloid fraction of the cactus (NA1, NA2, and NA3, see text for more details). Dashed and dotted lines indicate mean control values of larval and total viabilities, respectively. Asterisks denote significant differences with respect to the corresponding control ($P < 0.05$ in Dunnett tests, see text for more details).

Table 2. ANOVA and MANOVA testing for wing size and shape differences, respectively, considering a model with treatment and sex as fixed factors: degrees of freedom (d.f.), mean squares (MS), and statistic value (*F*)

Trait	Sources of variation		d.f.	MS	*F*
Wing size	Treatment		6	1 571 357.36	61 374.99**
	Sex		1	5 981 081.92	233 612.61**
	Treatment × sex		6	1 236 025.15	48 277.39**
	Error		355	25.60	
		Effect d.f.	Error d.f.	Wilks' value	*F*
Wing shape	Treatment	96	1933.08	0.47	2.88**
	Sex	16	340.00	0.95	1.07
	Treatment × sex	96	1933.08	0.42	3.31**

**$P < 0.001$.

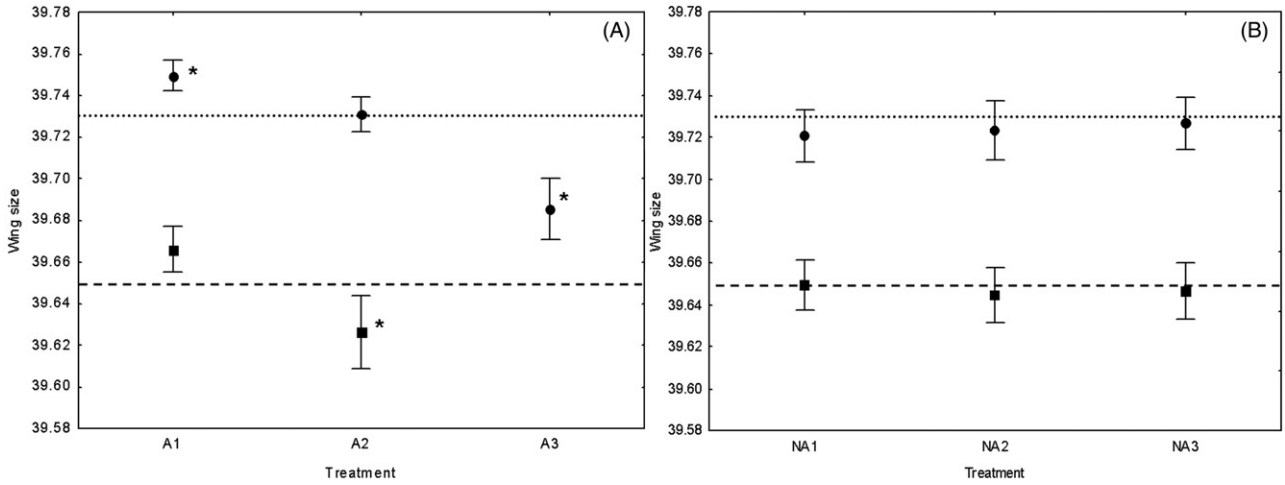

Figure 3. Mean wing size (expressed as the logarithm of centroid size) of *Drosophila buzzatii* males (squares) and females (circles) raised in: (A) standard laboratory medium plus increasing concentrations of the alkaloid fraction isolated from *Trichocereus terscheckii* (A1, A2, and A3, see text for details); and (B) standard laboratory medium plus increasing concentrations of the non-alkaloid fraction of the cactus (NA1, NA2 and NA3, see text for details). Dashed and dotted lines represent the mean values of males and females in control vials, respectively. Asterisks denote significant differences with respect to the corresponding control ($P < 0.05$ in Dunnett tests, see text for more details).

females exposed to the lowest concentration of the alkaloid extract (A1) had larger wings than control females, and that wings of males and females treated with the intermediate (A2) and highest (A3) concentrations of the alkaloid extract, respectively, were significantly smaller than controls (Fig. 3a). In contrast, the average wing size of flies raised in vials with different concentrations of the non-alkaloid fraction did not differ significantly from controls (Fig. 3b; all Dunnet's comparisons with $P > 0.05$).

Regression analyses revealed a negative and significant relationship between female wing size and concentration of the alkaloid extract (slope = –0.29, $R^2 = 0.32$, $P < 0.001$), but not with the concentration of the non-alkaloid fraction (slope ≈ 0, $R^2 = 0.006$, $P > 0.5$).

Regarding wing shape, flies exposed to different treatments showed significant differences relative to the control in both sexes for the two principal relative warps ($P < 0.005$ for all paired comparisons, error-corrected for multiple comparisons, Fig. 4). The landmarks involved in such differences correspond to a relatively small area circumscribed to the distal posterior wing region (Fig. 4). Additionally, we observed that the greater the concentration of the alkaloid or non-alkaloid fractions the larger the differences

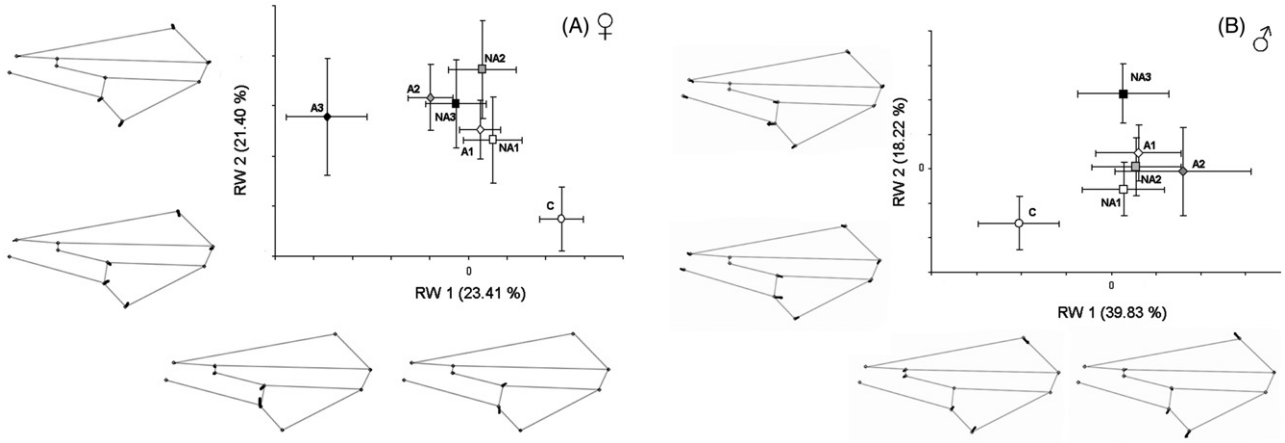

Figure 4. Mean wing shape (represented by the first two relative warps that explain the largest percentage of total wing shape variation), and standard deviation in females (A) and males (B) raised in standard laboratory medium (control represented by circles) and standard medium plus increasing concentrations of the alkaloid and non-alkaloid fractions extracted from *Trichocereus terscheckii* (diamonds and squares represent three different concentrations of the alkaloid and non-alkaloid fractions, respectively, see text for more details). Arrows indicate the magnitude and direction of landmark displacements with respect to the control. Arrow sizes have been magnified ten times to show wing-shape changes more clearly.

between treated and control flies. This is particularly evident in females after A3 treatment (Fig. 4a) and males of NA3 treatment (Fig. 4b) relative to the control.

Identification of the main basic compounds in the crude alkaloid fraction

Thin layer chromatography (TLC) was performed with the crude alkaloid fraction, as it was the biologically active fraction that impaired viability and reduced wing size. Only one study of *T. terscheckii* allelochemicals reported the presence of mescaline and other related compounds in some fractions with high alkaloid content (Reti & Castrillón, 1951). For this reason, we decided to perform an extra step in the identification of known basic compounds (present in the crude alkaloid fraction).

The mass spectrum confirmed the presence of two main basic compounds in *T. terscheckii*, which have been reported previously (Reti & Castrillón, 1951). One of them was mescaline (see fragmentation features in Ogunbodede *et al*., 2010) and the other was described as a novel vegetable base called trichocereine (*N*-dimethylmescaline; Fig. 5). The latter was reported as a new natural phenylethylamine alkaloid particular to this species (Reti & Castrillón, 1951). We also detected the mescaline analogue α-methylmescaline (Fig. 5; Hardman, Haavik & Seevers, 1973). These results confirm that the bioactive alkaloid fraction is enriched in phenylethylamines and mescaline-related compounds; however, it should be noted that other bases may be present in small amounts as the GC was not fully resolved.

DISCUSSION

The present study represents a first assessment of the chemical determinants resulting in reduced performance of *D. buzzatii* in the columnar cactus host *T. terscheckii*. We demonstrated that an alkaloid fraction extracted from this host negatively affected performance by reducing viability and decreasing wing size. These results support the hypothesis that cactus chemistry plays a relevant role in shaping patterns of host use in nature.

Our present results along with previous field and laboratory studies in *D. buzzatii* and allied species of the *D. buzzatii* cluster emphasize the remarkable influence that cactus hosts impose on the life history of these flies (Fanara *et al*., 1999; Fanara & Hasson, 2001; Carreira *et al*., 2006; Soto *et al*., 2007, 2012; Hasson *et al*., 2009). For example, 90% of the *Drosophila* that emerge from rotting cladodes of prickly pears (*Opuntia* spp.) are *D. buzzatii*, which also emerge, though only marginally, from rotting columnar cacti (10–40%, depending on the species and locality) of the genera *Echinopsis* or *Cereus* (Hasson *et al*., 1992). Actually, columnar cacti are the preferred hosts of the closely related species *Drosophila koepferae* Fontdevila & Wasserman, 1988 (Hasson *et al*., 1992, 2009; Soto *et al*., 2012). Viability, body size, developmental rate, starvation resistance, and mating success are maximized when flies develop in

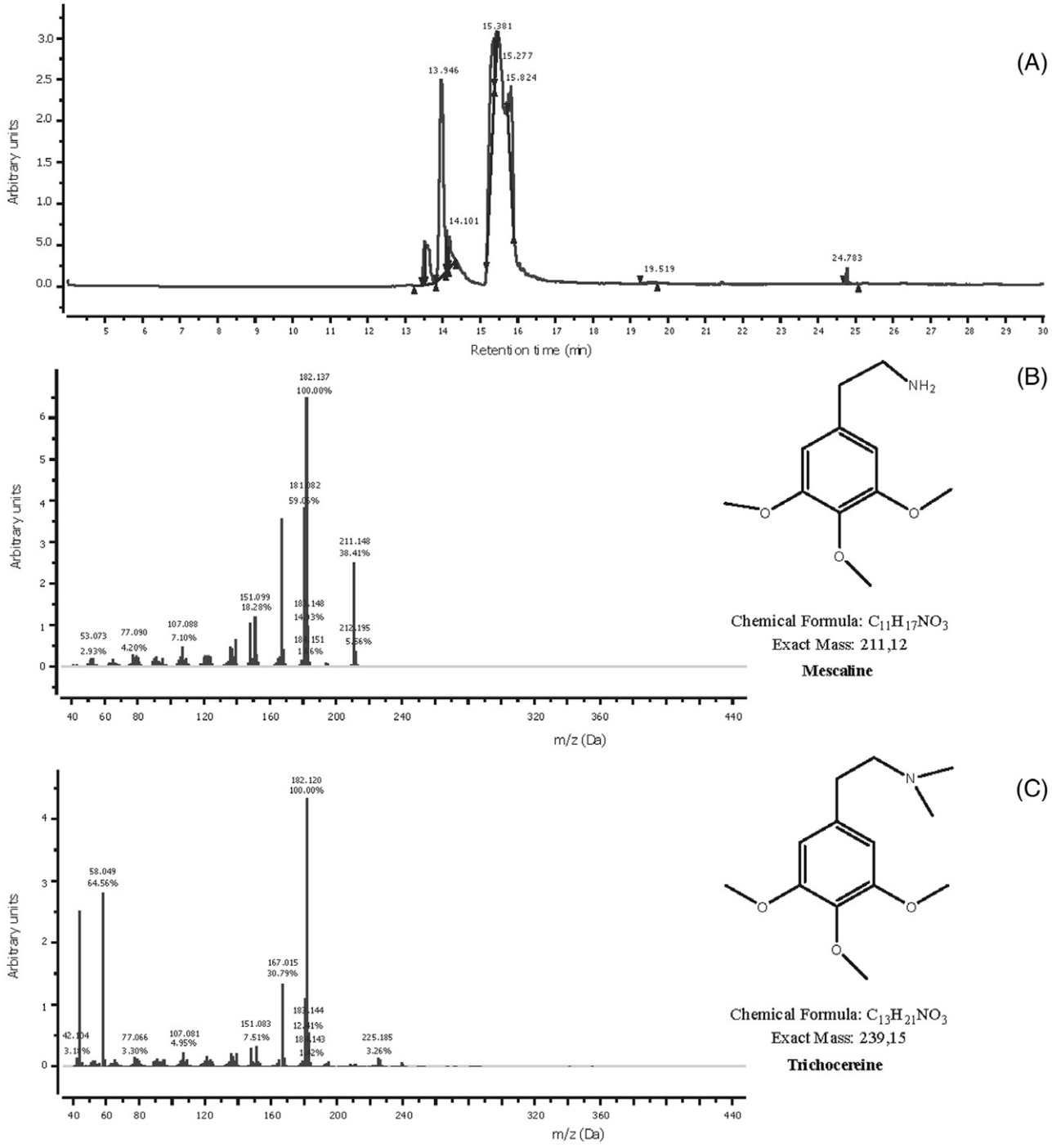

Figure 5. A, gas chromatography of the basic alkaloid extract of *Trichocereus terscheckii*. Confirmation by mass spectrum of the three main phenylethylamines: B, mass spectrum at a retention time of 15.42 min, corresponding to mescaline; C, mass spectrum at a retention time of 15.87 min, corresponding to trichocereine; D, mass spectrum at a retention time of 15.82 min, corresponding to α-methylmescaline. Note the characteristic mass peaks as well as the characteristic fragments of each molecular ion.

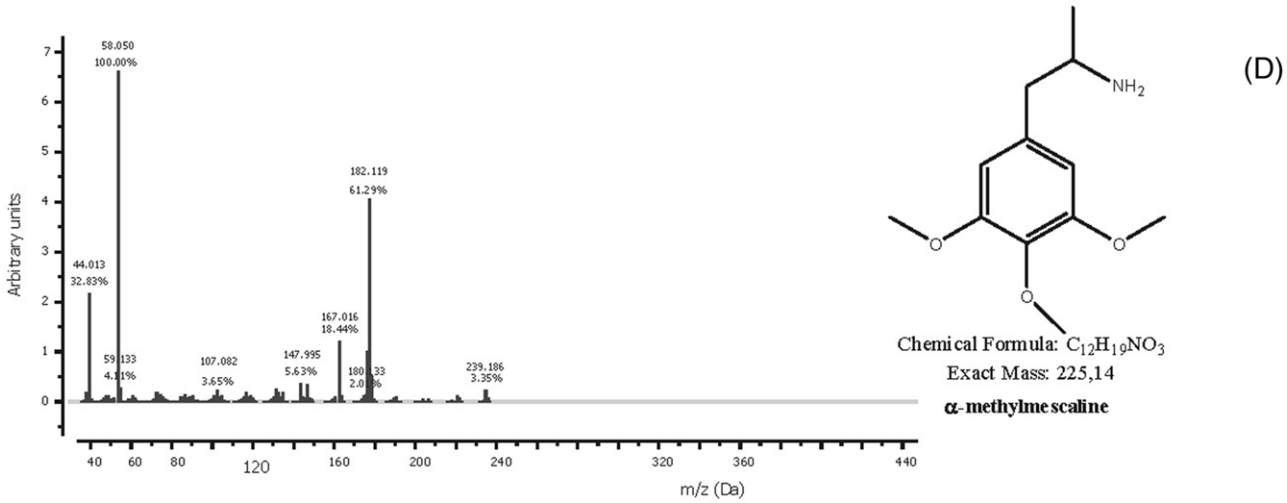

Figure 5. *Continued*

the prickly pear *O. sulphurea*, as compared with flies reared in *T. terscheckii* (Hasson *et al*., 2009; Hurtado *et al*., 2012; Soto *et al*., 2012), indicating that columnar cacti and prickly pears represent dissimilar challenges to the growing larvae, and that selection of the egg-laying substrate by a female may be crucial to the success of her progeny. Actually, *D. buzzatii* females prefer to oviposit on rotting tissues of the prickly pear *O. sulphurea* than on T. terscheckii (Fanara & Hasson, 2001; Soto *et al.*, 2012), in agreement with the expectations of the preference–performance or mother knows best hypothesis (reviewed in Craig & Itami, 2008; Gripenberg *et al.*, 2010; see also Soto *et al.*, 2012).

Patterns of differential performance in primary versus secondary hosts have been linked to ecological and chemical aspects of the host plants (Fanara *et al*., 1999; Hasson *et al*., 2009). Specifically, the presence (in columnar cacti)/absence (in prickly pears) of alkaloids has been proposed as a possible factor (Hasson *et al*., 2009; Soto *et al*., 2012).

Pre-adult viability is one of the best indicators of the degree of adaptation of an organism's physiological and genetic mechanisms to exploit a certain kind of resource, reflecting the ability to extract nutrients and/or eliminate toxic compounds (Hoffmann & Parsons, 1993; Hasson *et al*., 2009). Pre-adult viability in flies reared in *T. terscheckii* (Fanara *et al*., 1999) is, on average, greater than in laboratory medium plus the alkaloid fraction, and is fairly similar to the estimates obtained in laboratory medium plus the non-alkaloid fraction (this work). Also, the time needed to reach the adult stage (developmental time) is an important pre-adult life-history trait, as an increase of developmental time may be the first symptom of a noxious effect of plant secondary compounds on fitness in phytophagous insects. Developmental time was not directly measured in our experiment; however, there is evidence that flies start to emerge nearly simultaneously in control, non-alkaloid, and low-alkaloid concentration vials, whereas a 24-h delay and an emergence window that was extended for nearly 48 h, compared with controls, were observed in vials with high alkaloid concentration (I. Soto, C. Corio, and E. Hasson, unpubl. data). Overall, these results point to a noxious effect of *T. terscheckii* alkaloids on pre-adult life.

The partition of total viability in larval and pupal viability allowed us to determine the stage in which alkaloids caused the most toxic effect. Although the number of larvae that reached the pupal stage varied slightly across treatments, the alkaloid fraction apparently caused its main effect during the transition from the pupal to the adult stage, pointing to a failure in metamorphosis, an effect that was more pronounced in vials with the highest alkaloid concentration. As larvae tended to pupate outside the rearing medium (in vial walls or in the cotton cap), it may be argued that the failure of metamorphosis was not caused by direct contact of pupae with the medium, but was more likely to have been caused by the accumulation of a toxic compound during larval growth. These results suggest that growing in an alkaloid-rich food may damage developmental functions during metamorphosis, in line with work in other insects such as the silk moth *Philosamia ricini* (Donovan, 1798) (Narberhaus, Zintgraf & Dobler, 2005).

Adult morphology was also affected by the presence of alkaloids in the rearing medium. *Drosophila buzzatii* reared in *T. terscheckii* have smaller thorax length and wing size than flies raised in prickly pear,

which was partially attributed to the putative detrimental effect of alkaloids (Soto *et al*., 2008; Hasson *et al*., 2009). In the present study, we provide direct support for the hypothesis that increasing alkaloid concentration reduces wing size. We also showed that the presence of alkaloids in the rearing medium resulted in changes in wing shape. Specifically, the region of the wing exhibiting the most change was an intervein region known to be involved in a genotype–rearing cactus interaction (Soto *et al*., 2008).

Interestingly, viability in vials with the highest concentration of the non-alkaloid fraction was higher than in the controls. Even though the alkaloid fraction was *a priori* our first candidate to account for the effect of *T. terscheckii* on fly fitness, we also included the non-alkaloid fraction in a set of separate treatments because it is expected to contain a complex mix of acid liposoluble compounds (terpenoids, fatty acids, sterols, and aromatics). However, the non-alkaloid fraction of *T. terscheckii* did not cause detrimental effects in *D. buzzatii*, ruling out the presence of toxic liposoluble compounds. In contrast, liposoluble triterpene glycosides isolated from *Stenocereus thurberi* (Engelm.) Buxbaum and from *Stenocereus gummosus* (Engelm.) Gibson & Horak affected larval viability, extended developmental time, and reduced body size in *Drosophila mojavensis* Patterson & Crow, 1940 (Fogleman & Armstrong, 1989). Furthermore, the lipidic fractions of *S. thurberi* and *S. gummosus* were also found to contain unusual medium-chain fatty acids and sterols that are toxic to most *Drosophila* species inhabiting the Sonora Desert, except for *D. mojavensis* (Fogleman & Danielson, 2001).

Overall, our studies show that *T. terscheckii* and the presence of alkaloids in the rearing medium impose stressful conditions to larvae during development (Soto *et al*., 2008; Soto *et al*., 2012; Hasson *et al*., 2009; present work), suggesting that host-plant shifts have been a relevant factor in the evolution of the *D. buzzatii* cluster. Moreover, it may be argued that host shifts for prickly pear dwellers like *D. buzzatii* to the more hostile environment offered by columnar cacti are more stressful than for the other species of the *D. buzzatii* cluster, which are mainly columnar dwellers (Manfrin & Sene, 2006; Hasson *et al*., 2009). Yet, shifts from prickly pear, which are considered the most likely ancestral host, to columnar cacti (and vice versa) have occurred several times in the evolutionary history of the repleta group (Oliveira *et al*., 2012). Particularly dramatic are the cases of *Drosophila pachea* Patterson & Wheeler, 1942 and *Drosophila mettleri* Heed, 1977 (reviewed in Fogleman & Danielson, 2001). The former is tolerant to very high concentrations of alkaloids and restricted to the necroses of *Lophocereus schottii* (Engelm.) Hunt (senita) because of an absolute requirement of certain sterols not found in other cacti. This strict dependence of *D. pachea* on *L. schotii* is because of several amino acid changes in the gene *neverland oxygenase* (Lang *et al*., 2012). *Drosophila mettleri* breeds and feeds in soils soaked with exudates from rotting saguaro (*Carnegiea gigantea* Britton & Rose; saguaro), where alkaloid concentrations may be up to 20-fold greater than in fresh cactus. Thus, we hypothesize that host switches to columnar cacti are accompanied by adaptations increasing tolerance to toxic alkaloids triggering the rapid evolution of detoxification mechanisms (Amlou, Moreteau & David, 1998; Etges *et al*., 2006; Matzkin *et al*., 2006). Because several independent shifts to columnar hosts have been identified in the evolutionary history of the repleta group (Oliveira *et al*., 2012), this system provides a unique opportunity to investigate the repertoire of genes (and/or alleles) and metabolic pathways that facilitate the exploitation of alternative types of resources.

Finally, it is important to note that as the alkaloid extract used may impose severe conditions to the growing larvae that may not be representative of what flies experience in nature, our results should be interpreted with caution. In addition, the microorganisms (yeasts and bacteria) involved in the rotting process of cactus tissues often modify the chemistry of the host and reduce its toxicity, and these were not considered in the experimental design. Therefore, future experiments will involve the identification of the specific alkaloid, or mixture of compounds, responsible for the biological effects observed in *D. buzzatii* as well as the role of the microflora associated with the rotting process.

In the present study we have accomplished the first step in the identification and testing of candidate compounds related to host toxicity in *D. buzzatii*. Subsequent studies will focus on evaluating the biological effect of specific alkaloids (e.g. tyramine, *N*-methyltyramine, hordenine, and candicine) known to be present in other columnar cacti of the same genus (Gibson & Nobel, 1986), and that have been associated with toxic effects in other *Drosophila* species (Geber, 1967; Hardman *et al*., 1973; Hirsh and Fritz, 1981).

ACKNOWLEDGEMENTS

The authors wish to thank E. Soto for helpful comments in the early stages of this investigation, and J. Palermo, S. Szajnman, and M. Sánchez for invaluable technical assistance. We also thank two anonymous reviewers for insightful comments and helpful advice. We also wish to express our appreciation to Maxi Richmond for critical reading, comments, suggestions, and corrections of the English grammar.

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), and Universidad de Buenos Aires. J.P. and M.I.L.B. are postgraduate fellows of CONICET. V.P.C., I.M.S., and E.H. are members of Carrera del Investigador Científico (CONICET).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website:

Table S1. Mean viability (and standard error) for each treatment (A1–A3 and NA1–NA3 represent three different concentrations of the alkaloid and non-alkaloid fractions, respectively; ten replicates each, see text for details). Pupal mortality was calculated as the difference between total and larval viability. Entries in bolded differed significantly from the control ($P < 0.05$ in Dunnett tests, see text for more details).

Table S2. Mean wing size (expressed as the logarithm of centroid size) and standard error (between parentheses) for each treatment (A1–A3 and NA1–NA3 represent three different concentrations of the alkaloid and non-alkaloid fractions, respectively; see text for details) and sex.