

¹Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina; ²Departamento de Biología, FFCLRP-USP, Ribeirão Preto-SP, Brazil

Host-dependent phenotypic plasticity of aedeagus morphology in a pair of cactophilic sibling *Drosophila* species of the *repleta* group (Diptera, Drosophilidae)

I. M. SOTO¹, M. H. MANFRIN² and E. HASSON¹

Abstract

The rapid evolution of male genital morphology is a characteristic feature of several animal groups. Such rapid divergence makes this trait a useful key for species identification. The aedeagus, the intromittent organ of male genitalia, is considered the main diagnostic trait in the *Drosophila repleta* group. In this study we analysed phenotypic plasticity and genetic variations associated with aedeagus size and shape in the cactophilic sibling species *Drosophila gouveai* Tidon-Sklorz and Sene, 2001 and *Drosophila antonietae* Tidon-Sklorz and Sene, 2001. Phenotypic plasticity in aedeagus morphology was evaluated in terms of the response to rearing media prepared with each species' natural host plant, *Pilosocereus machrisii* Dawson, 1957 and *Cereus hildmannianus* Schum, 1890 respectively. Our results show that aedeagal shape differed significantly between species and that both shape and size presented host-related phenotypic plasticity in both species. Flies reared on *P. machrisii* had, on average, larger aedeagi than those grown in *C. hildmannianus*. The general shape of aedeagus also differed significantly between flies that emerged in different host cactus. Patterns of variation in aedeagus morphology are discussed in the light of the current knowledge of evolutionary relationships and host plant use, in the *D. buzzatii* cluster, an assemblage of species in active cladogenesis.

Key words: aedeagus – cactus – Fourier descriptors

Introduction

The rapid evolution of genitalic traits, particularly those that are male-specific, makes this kind of traits useful keys for species identification in animal groups with internal fertilization (Eberhard 1993; Arnqvist 1997). However, the mechanisms and processes involved in male genitalic divergence are far from being understood and are a matter of debate among evolutionary biologists (Hosken and Stockley 2004).

Three main hypotheses have been proposed to account for the rapid evolution of genital morphology: lock-and-key (Dufour 1844), pleiotropy (Mayr 1963) and sexual selection (Eberhard 1993). The lock-and-key hypothesis considers that male genitalia (the lock) evolves as a species-specific trait and is constrained to properly fit in female genitalic organs (the lock). This hypothesis predicts both limited phenotypic variation and low levels of genetic variance in genital morphology (Shapiro and Porter 1989; Arnqvist 1997). The pleiotropy hypothesis assumes that genital variation is largely neutral, and as genital traits are implicitly assumed to be genetically correlated with other non-genitalic traits, changes in allele frequencies at pleiotropic loci affecting both general morphology and genital morphology may lead to rapid and even arbitrary evolution of genital traits. Finally, the hypothesis of sexual selection predicts a correlation between morphological variation in male genitalia and fertilization success via male–male competition or female choice (Arnqvist 1997). Nowadays, explanations excluding sexual selection from the discussion of the evolutionary forces and actual targets of selection involved in genital evolution have lost support (reviewed in Hosken and Stockley 2004).

In the genus *Drosophila* and particularly in the *Drosophila repleta* group the morphology of the aedeagus is a diagnostic trait that provides, along with chromosomal inversions, a guide for species identification (Vilela 1983). The *Drosophila buzzatii* cluster (*D. repleta* group) is a Neotropical group of seven species inhabiting the arid and semiarid lands of

southern South America. All species breed and feed on the necrotic tissues of several genera of Cactaceae (Manfrin and Sene 2006). In this group, the genitalia is the only key trait that allows to distinguish sibling species, as females of both the sibling species are morphologically similar. Evolutionary relationships inferred on the basis of aedeagus morphology divide the *D. buzzatii* cluster in two main clades. One is monospecific and includes *D. buzzatii* Carson and Wasserman, 1965, which exhibits qualitative and quantitative differences in aedeagus morphology when compared with the remaining species (see Fig. 3 in Manfrin and Sene 2006). The second clade, known as the *Drosophila serido* sibling set, includes the other six species which exhibit quantitative differences in aedeagus morphology (Tidon-Sklorz and Sene 2001). In fact, five species of the *D. serido* sibling set (*Drosophila serido* Vilela and Sene, 1977; *Drosophila koepferae* Fontdevila et al., 1988; *Drosophila seriema* Tidon-Sklorz and Sene, 1995; *Drosophila antonietae* Tidon-Sklorz and Sene, 2001; and *Drosophila gouveai* Tidon-Sklorz and Sene, 2001) had been initially considered as members of the same species, *D. serido*, because of their close similarity in aedeagus morphology (Vilela and Sene 1977). However, phylogenetic relationships inferred on the basis of molecular and morphological evidence are in partial conflict, suggesting that evolutionary relationships in the *D. buzzatii* cluster constitute an evolutionary conundrum (Manfrin et al. 2001; Soto et al. 2007a).

Drosophila gouveai and *D. antonietae* are two closely related species that belong to the *D. serido* sibling set (Manfrin et al. 2001; Manfrin and Sene 2006). The former is distributed from mid-western Brazil to the southern boundary of the Cerrado Domain (Tidon-Sklorz and Sene 2001). In the southern section of its geographic range, *D. gouveai* is found in sandstone table hills associated with the necroses of the columnar cactus *Pilosocereus machrisii* (Monteiro and Sene 1995). In the north and north-east regions of its distribution, it occurs in large areas with great cactus diversity including several species of the

genera *Pilosocereus* and *Cereus* (Pereira et al. 1983). *D. antonietae* (Tidon-Sklorz and Sene 2001) is found in south and south-eastern Brazil and north of the eastern border of the Argentinian Chaco, where it is mainly associated with the columnar *C. hildmannianus* Schum, 1890 and occasionally with *Opuntia monacantha* Willdenow, 1813 (Manfrin and Sene 2006).

Current evidence suggests that the southern limit of the distribution of *D. gouveai* is very close to the northern limit for *D. antonietae*. In this region *C. hildmannianus* and *P. machrisii* grow close together inside an east–west corridor of approximately 200 km (Manfrin and Sene 2006). Evidence of possible (past or present) introgression events has been found in this area (Manfrin et al. 2001; De Brito et al. 2002).

The main objective of this study was to understand the factors involved in aedeagal evolution in pairs of closely related species of the *D. buzzatii* cluster in different stages of divergence. To this end we investigate sources of phenotypic variation, genetic and environmental, in aedeagus morphology by examining its size and shape in *D. gouveai* and *D. antonietae*, reared in the major host cacti that each species utilize in nature as feeding and breeding resources.

Materials and Methods

Experimental design

Four isofemale lines of both *D. gouveai* and *D. antonietae* were analysed in the present study. These stocks were founded with the progeny of naturally inseminated females collected in different localities from Brazil (sites of collection detailed in Manfrin et al. 2001). Flies were reared in identical conditions in bottles with 30 ml of *Drosophila* instant medium (Carolina Biological Supplies, Burlington, NC, USA) for several generations and were never exposed to the media prepared with rotting cactus before the experiments described below. We also collected fresh cactus and the juice exuded from naturally occurring rotting cacti of both *C. hildmannianus* and *P. machrisii* in the sampling localities. Pieces of fresh cactus were stored at -20°C and the fermenting juices maintained in the laboratory by adding 10 g of fresh cactus every 2 weeks. Strains were maintained and experiments were performed under controlled temperature and light regimes [$25 \pm 1^{\circ}\text{C}$; 12 : 12 h (L : D) photoperiod].

One hundred pairs of sexually mature flies were released in egg-collecting chambers (two chambers per isofemale line) containing a Petri dish with an egg-laying medium. Petri dishes were removed 12 h later and incubated at 25°C until egg hatching. Batches of 30 first instar larvae were collected and seeded in vials containing 6 ml of one of the two cactus media prepared with experimentally rotten pieces of *P. machrisii* or *C. hildmannianus*.

For the preparation of the cactus media, pieces of cactus were ground in a blender and 6 g poured into glass vials. Afterwards, vials were inoculated with 0.1 ml of the fermenting juice of the corresponding cactus species and stored at 25°C for 24 h before seeding the larvae (see Fanara et al. 1999 for a thorough description of the methodology).

Four replicate vials were used for each combination of isofemale line and cactus, and periodically checked until adult emergence. Adult flies were simultaneously collected and sexed under light CO_2 anaesthesia. Emerged males were allowed to reach sexual maturity and their aedeagi were removed and mounted on slides. Slides were photographed with a digital camera mounted on a microscope at $400\times$ magnification.

Morphological quantification

The aedeagus of both species analysed is laterally flattened with two hemi-pieces fused in their median dorsal margin. Thus, it can be effectively described in lateral view as a bidimensional structure (Soto 2005; Soto et al. 2007a). In the quantification of the contour, only the

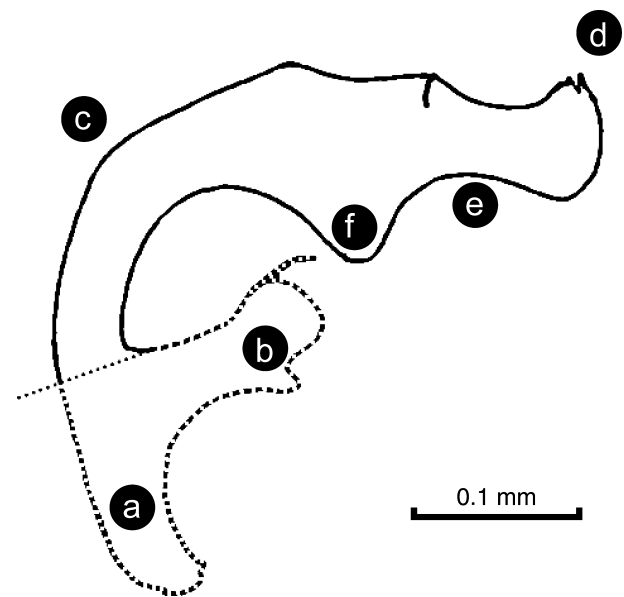


Fig. 1. Diagrammatic sketch in lateral view of a typical aedeagus showing the location of the parts referred to in the text (a–f). The dotted outline represents the portion of the organ excluded in the quantification of size and shape variation. (a) Aedeagal apodeme; (b) paraphysis; (c) dorsal margin; (d) tip; (e) distal ventral margin; (f) ventral process

structure effectively involved in the penetration of female genitalia, i.e. the aedeagus itself, therefore excluding the aedeagal apodeme and paraphysis (Fig. 1; see Vilela 1983 for details of the morphology of male genitalia), was considered.

We employed elliptic Fourier descriptors (EFDs) (Kuhl and Giardina 1982) for shape quantification. Differences in x and y coordinates of an outline were fitted separately as functions of arc length by Fourier analysis. Thus, the outline was decomposed into a weighted sum of sine and cosine functions designated as harmonics. Fourier coefficients for a polynomial function of 30th degree were obtained from the outlines of digital images using SHAPE v1.2 package (Iwata and Ukai 2002). The lateral area of each aedeagus (measured on the digital images and expressed in pixels) was considered as an estimator of the size of the organ. Then, for shape analyses, size, orientation and starting position of the contours were standardized in accordance with the size and alignment of the major axis of the first ellipse, obtaining representations of the organs that were only based on internal properties of the outlines (i.e. shape) (Kuhl and Giardina 1982).

We performed a principal component analysis of the variance–covariance matrix of the 120 (4 per harmonic) estimated coefficients. This procedure allowed us to summarize the information assessed in the coefficients and reduce the dimensionality of the variables (Rohlf and Archie 1984) in a lower number of principal components (PC). The resulting scores of each PC of each specimen were considered as reorganized uncorrelated morphological traits representing different aspects of total shape variation (Iwata and Ukai 2002). These variables were further used as shape descriptors in subsequent analyses.

Analysis of variation in aedeagus size and shape

Size differences were investigated by means of a mixed ANOVA model with host cactus and fly species (two levels each, fixed) and isofemale line (four levels, nested in species) as the main sources of variation. Individuals that emerged in different vials of the same isofemale line (replicates) were pooled under the line factor. The variable was log-transformed to ensure homoscedasticity. Shape variation was assessed by means of a MANOVA using PC scores as dependent variables and the same independent factor used in size analyses.

According to our experimental design, a significant cactus effect (C) may be interpreted as phenotypic plasticity, while significant differences among isofemale lines (L) as due to genetic differences (as all lines were reared under the same conditions). Finally, a significant $L \times C$ interaction may be interpreted as a genotype-by-environment interaction (GEI), or in other words, that the responses of isofemale lines are not independent of the rearing cactus. Statistica 6.0 (StatSoft, Inc 2001) was used for statistical analyses and in all cases the corresponding assumptions were properly tested.

Results

A total of 90 *D. gouveai* and 93 *D. antonietae* males were analysed in this study. The total number of PCs explaining a significant portion of shape variation was 15, that jointly accounted for 91.6% of total shape variation in the original variance-covariance matrix. The morphological variation summarized by the first eight PCs of the elliptic Fourier descriptors accounted for nearly 83% of total shape variance (Fig. 2).

The ANOVA testing for differences in aedeagus size showed a consistent cactus effect (Table 1a). Males of both species grown in *P. machrisii* had a greater aedeagus size than those raised in *C. hildmannianus*. Variation in male aedeagus size, in

response to the rearing media, was species-specific (cactus-by-species interaction, Fig. 3). Interestingly, *a posteriori* comparisons between species showed that size differences were not significant either in *C. hildmannianus* or *P. machrisii* ($p = 0.379$ and 0.365 respectively), suggesting that the significant cactus-by-species interaction might be accounted for by a lack of parallelism between the reaction norms of each species and could only be noticed when considering the entire data set. Differences among isofemale lines (within species) were also significant. According to our experimental design a significant line effect suggests that an important fraction of variation in size may be due to genetic differences among families. The lack of interaction between line and cactus (Table 1a) suggests that the reaction norms (plastic responses) of different lines (families or genotypes) were independent of the rearing cactus.

In the case of the shape of the aedeagus, differences between species and between flies reared in different cactus hosts were significant; however, the reaction norms of both species were parallel (Table 1b). In Fig. 4 we present a plot of the first two principal components (PC1 and PC2), that together account for nearly 60% of total shape variance. The combination of both PC scores allows us to discriminate between species and

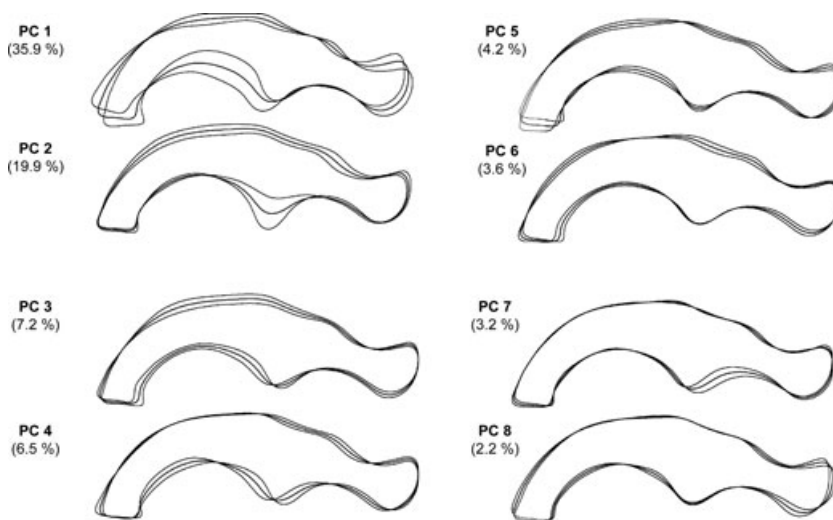


Fig. 2. Outlines representing patterns of interspecific shape variation aedeagus shape accounted (percentage in parentheses) by the first eight principal components. Each outline was reconstructed from the estimated coefficients by letting the score of the corresponding principal component to be equal to the mean and mean plus or minus two standard deviations (SD) and the remaining components set to zero. Lines stand for mean shape, +2 SD and -2 SD

Table 1. (a) ANOVA and (b) MANOVA testing for size and shape variation in aedeagus morphology, in *D. antonietae* and *D. gouveai*

Trait	SS	d.f.	MS	d.f. error	MS error	F-value
<i>(a) Size</i>						
Species (SP)	0.31	1	3.12	7.11	27.60	0.11
Cactus (C)	2.84	1	28.44	15.13	6.00	4.74*
SP \times cactus	2.90	1	28.99	15.13	6.00	4.83*
Line (SP)	19.94	6	33.23	6	4.92	6.75*
Cactus (C) \times line (SP)	2.95	6	4.92	167	9.47	0.52
Error	158.20	167	9.47			
		Wilk's value	d.f. effect	d.f. error		F-value
<i>(b) Shape</i>						
Species (SP)		0.47	15	153		11.4**
Cactus (C)		0.83	15	153		2.1*
SP \times cactus		0.89	15	153		1.1
Line (SP)		0.23	90	867.025		2.9**
Cactus (C) \times line (SP)		0.44	90	867.025		1.5**

d.f., degrees of freedom; SS, sum of squares; MS, mean squares.

* $p < 0.01$; ** $p < 0.001$.

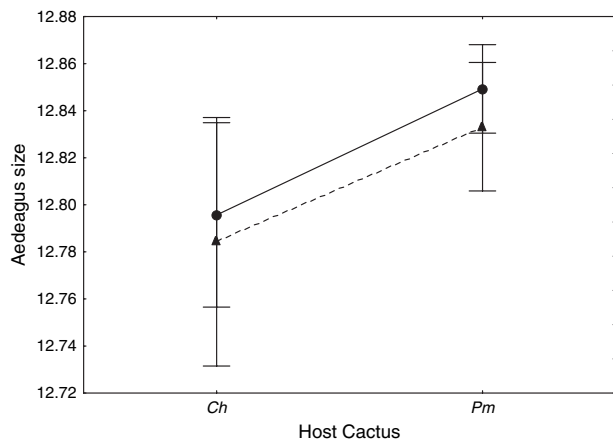


Fig. 3. Phenotypic plasticity in genital size. Mean aedeagus size (log of number of pixels of organ image, see Materials and methods for details) and 95% confidence intervals of *D. antonietae* (triangles) and *D. gouveai* (circles) raised in *Cereus hildmannianus* (Ch) or *Pilosocereus machrisii* (Pm)

even between flies that emerged in different cactus hosts. Interestingly, host-related plastic changes and changes associated with interspecific divergence in aedeagus shape involved the same portions of the organ. In effect, variation in shape involved changes in the dorsal margin ('c' in Fig. 1) of the organ (increasing values of PC1 were associated with a more curved dorsal margin) and the ventral process in the ventral margin ('e' in Fig 1) (increasing values of PC2 were related with a decrease in the relative contribution of this portion to total outlined shape). *D. gouveai* males raised in *P. machrisii*, its putative preferred host plant (Manfrin and Sene 2006), tended to have a more curved dorsal margin than *D. antonietae* males reared in *C. hildmannianus*, while *D. gouveai* males grown in *C. hildmannianus* tended to have a less pronounced ventral process than *D. antonietae* reared in *P. machrisii* (Fig. 4). Differences among lines (within species) and the line-by-cactus interaction were also significant (Table 1b). Overall, these results suggest that the reaction norms (plastic responses)

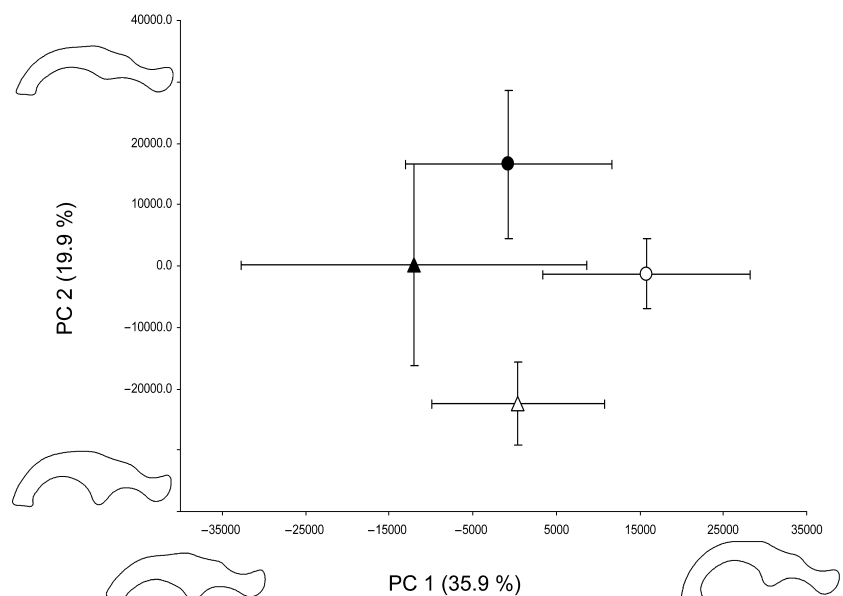
of isofemale lines were not independent of the cactus host, or in other words, a significant genotype-by-environment interaction in the shape of the aedeagus.

Discussion

In phytophagous insects, host plants are a key biotic factor, which affects several morphological and physiological characters (Etges 1990; Hawthorne and Via 2001; Dambroski et al. 2005). Particularly in the *Drosophila buzzatii* cluster, there is abundant evidence showing that the morphology and performance (viability and developmental time) of flies are differentially affected by the rearing cactus hosts (Fanara and Hasson 2001; Soto et al. 2007a,b,c). In this sense, our study shows that aedeagus morphology (both size and shape) is affected by the rearing cactus.

One important point raised by the comparison between *D. gouveai* and *D. antonietae* is that differentiation between species varied across portions of the aedeagus. This result suggests a certain degree of heterogeneity in the degree of the evolutionary constraints among parts of the intromittent organ. Genital differences between *D. gouveai* and *D. antonietae* were more conspicuous in the posterior dorsal margin from the fused portion to the tip of the aedeagus and the ventral process (affecting arcs II and III, according to Fig. 1 of Silva and Sene 1991). In addition, it is interesting to note that the most variable parts of the organ identified in the interspecific comparison were also those that exhibited the most prominent plastic responses. In effect, plasticity was apparent within species and the most prominent changes involved the distal part that altered the proportions of arcs II and III. In general terms, adult males that grew in *P. machrisii* had larger aedeagus than those grown in *C. hildmannianus*. Regarding shape, males grown in *P. machrisii* had a more elongated, twisted and conspicuous tip, and ventral process than those grown in the other cactus host. Therefore, an important conclusion of our study is that host plants exert an important effect on aedeagal morphology in both species, although interspecific differences are maintained by nearly parallel reaction norms.

Fig. 4. Phenotypic plasticity in genital shape. Plot of mean shape scores (and confidence intervals) of both species (triangles: *D. antonietae*; circles: *D. gouveai*) in both cactus hosts (filled symbols, *Cereus*; empty symbols, *Pilosocereus*) for the first two principal dimensions accounting shape variation (PC1 and PC2, in parenthesis the percentage of shape variance accounted). Outlines of aedeagi by each axis depict genital shape variation accounted by each principal component (representing 2 standard deviations from the mean shape)



Such sensitivity to cactus hosts is not an exclusive feature of the aedeagus. Actually, other traits such as wing morphology (Soto et al. 2007b) and fitness-related traits (Soto et al. 2007c) are also phenotypically plastic, if we compare the responses of flies reared in *P. machrisii* and *C. hildmannianus*. Suggestively, flies reared in *C. hildmannianus* were the most divergent from the overall mean, as also observed regarding wing size (Soto et al. 2007b), pointing out that this cactus certainly had detrimental effects on the developing flies, especially on *D. gouveai*. Our second conclusion is that patterns of morphological variation in size observed for the wing (Soto et al. 2007b) and aedeagus (this study), and for fitness traits (Soto et al. 2007c) suggest that growing in different cactus hosts may have several biological consequences that may affect flies' performance in nature. These observations emphasize the idea that *C. hildmannianus* and *P. machrisii* may be perceived as different breeding sites by *D. gouveai* and *D. antonietae*. Such host-dependent expression of morphology and life history traits have been also reported in *D. buzzatii* and *Drosophila koepferae*, another pair of closely related species of the *D. buzzatii* cluster (Fanara et al. 2004; Carreira et al. 2006).

Whether these features are a common characteristic of the *D. buzzatii* cluster as a group or may even be extended to the rest of the set of cactophilic species of the *repleta* group is a matter of speculation. However, studies of another guild of cactophilic species of the *repleta* group also indicate that the rearing cactus has a profound influence in the evolution of the *D. mojavensis* cluster (Etges 1993; Stennett and Etges 1997).

Environment-induced variations in male genitalia have also been reported in other insects. For example, seasonal variation in the morphology of male genitalia has been reported in the chironomid *Procladius choreus* (affected by seasonality, Kobayashi 1998). Likewise, temperature fluctuations during larval development also induce changes in male genitalia in the mosquito *Anopheles albimanus* (Hribar 1996). Arnqvist and Thornhill (1998) found that certain features of the morphology of the genitalia of the water strider *Gerris incognitus* are plastic and sensitive to conditions of feeding stress. In *Drosophila*, Andrade et al. (2005) found a plastic response of the morphology of the aedeagus to variation in temperature in *D. mediopunctata*. All in all, these studies indicate that male genital morphology is a phenotypically plastic organ in insects, arguing against the lock-and-key hypothesis. The results of a recent study of male genitalia in *D. buzzatii* and *D. koepferae* are also similar (Soto et al. 2007a).

Drosophila koepferae, *D. gouveai* and *D. antonietae* have very similar morphotypes of male genitalia (Tidon-Sklorz and Sene 2001). It may be argued that the morphological resemblance among species of the so-called *D. serido* sibling set, to which these species belong, might be attributed to their recent common origin related to an apparent absence of selective pressures that may lead to morphological differentiation, as all species share common environmental and ecological features in their niche consisting of the rotting pockets of different columnar cacti (Tidon-Sklorz and Sene 2001). How could we differentiate a phylogenetic restriction from the absence of a divergent selective force (whether natural or sexual selection) in a scenario of only quantitative morphological differences? One possible interpretation of these similarities is that they are a consequence of a shared ancestral genetic background that did not diverge substantially, illustrating a phylogenetic constraint. However, these three species showed qualitative differences in their patterns of phenotypic plasticity when

reared in different cacti, suggesting different degrees of canalization in the development of male genitalia (Soto et al. 2007a and this study).

Our concluding remark is that, on the one hand, our study helps disclose some aspects of the evolution of the morphology of male genitalia, and on the other, that it also opens several interesting questions. First, which are the implications of intraspecific variation in male genital morphology on reproductive success? Second, have host-related morphological differences any effect on male sexual performance or success or it is just a plastic response with no fitness consequences? Which are the genes governing the differential response of genital morphology in different rearing cacti? These are open questions that should be addressed in future studies in order to elucidate the evolutionary history of a group of species in active cladogenesis that diversified in association with the expansion of the arid zones in southern South America.

Acknowledgements

We are particularly grateful to P.R. Epifanio, A. Esguicero and F.F. Franco for sample collection and technical assistance. We wish to thank V.P. Carreira and E. Soto for insightful comments during the drafting of this manuscript. The authors wish to thank two anonymous reviewers and Prof. D. Sperlich for advice and critical comments that helped to improve earlier versions of this manuscript. This work was supported by ANPCyT, CONICET and Universidad de Buenos Aires (UBA) in Argentina and Universidade de Sao Paulo (USP), CNPq, FAPESP and FINEP in Brazil and a joint project CAPES/SECyT (Project 071/04). IMS is fellow of CONICET (Argentina) and EH is member of Carrera del Investigador Científico (CONICET).

Resumen

Plasticidad fenotípica dependiente del hospedador en la morfología genital masculina de especies hermanas de Drosophila cactófilas del grupo repleta (Diptera, Drosophilidae)

Los órganos genitales de los machos han evolucionado de manera rápida y divergente en varios grupos de animales con fertilización interna permitiendo su utilización como caracteres identificadores de especies. Dentro del grupo de especies *Drosophila repleta*, al órgano intromitente masculino, aedeago, se lo considera el principal carácter diagnóstico. Hemos analizado la plasticidad fenotípica del tamaño y conformación de aedeagos en un par de especies cactófilas, *D. gouveai* Tidon-Sklorz y Sene, 2001 y *D. antonietae* Tidon-Sklorz y Sene, 2001 como respuesta a la crianza en sus plantas hospedadoras naturales, *Cereus hildmannianus* Schum, 1890 y *Pilosocereus machrisii* Dawson, 1957. Las especies difirieron en la conformación de sus genitales. Además, tanto la conformación como el tamaño genital presentaron plasticidad fenotípica relacionada con el hospedador. Los aedeagos de los machos de ambas especies fueron más grandes cuando las moscas se criaron en el medio preparado con *P. machrisii* y tuvieron una conformación distinta de aquellos criados en *C. hildmannianus*. Los patrones de variación en morfología genital se discuten a la luz de actual conocimiento acerca de las relaciones evolutivas y la evolución de la utilización de plantas hospedadoras en el cluster *Drosophila buzzatii*, un agregado de especies en activa cladogénesis.

References

- Andrade CAC, Hatadania LM, Klaczko LB (2005) Phenotypic plasticity of the aedeagus of *Drosophila mediopunctata*: effect of the temperature. *J Therm Biol* **30**:518–523.
- Arnqvist G (1997) The evolution of animal genitalia: distinguishing between hypotheses by single species studies. *Biol J Linn Soc* **60**:365–379.

- Arnqvist G, Thornhill R (1998) Evolution of animal genitalia: patterns of phenotypic and genotypic variation and condition-dependence of genital and non-genital morphology in a water strider (Heteroptera: Gerridae). *Genet Res* **71**:193–212.
- Carreira VP, Soto IM, Hasson E, Fanara JJ (2006) Patterns of variation in wing morphology in the cactophilic *Drosophila buzzatii* and its sibling *D. koepferae*. *J Evol Biol* **9**:1275–1282.
- Dambroski HR, Linn C, Berlocher SH, Forbes AA, Roelofs W, Feder JL (2005) The genetic basis for fruit odor discrimination in *Rhagoletis* flies and its significance for sympatric host shifts. *Evolution* **59**:1953–1964.
- De Brito RA., Manfrin MH, Sene FM (2002) Nested cladistic analysis of Brazilian populations of *Drosophila serido*. *Mol Phylogenet Evol* **22**:131–143.
- Dufour L (1844) Anatomie générale des Diptères. *Annuaire de Science Naturelle* **1**:244–264.
- Eberhard WG (1993) Evaluating models of sexual selection: genitalia as a test case. *Am Nat* **142**:564–571.
- Etges WJ (1990) Direction of life history evolution in *Drosophila mojavensis*. In: Barker JSF, Starmer WT, MacIntyre RJ (eds), *Ecological and Evolutionary Genetics of Drosophila*. Plenum Press, New York, pp. 37–56.
- Etges WJ (1993) Genetics of Host-Cactus Response and Life-History Evolution among Ancestral and Derived Populations of Cactophilic *Drosophila mojavensis*. *Evolution* **47**:750–767.
- Fanara JJ, Hasson E (2001) Oviposition acceptance and fecundity schedule in the cactophilic sibling species *Drosophila buzzatii* and *D. koepferae* on their natural hosts. *Evolution* **55**:2615–2619.
- Fanara JJ, Fontdevila A, Hasson E (1999) Oviposition preference, viability, developmental time and body size in the cactophilic sibling species *Drosophila koepferae* and *D. buzzatii* in association to their natural hosts. *Evol Ecol* **13**:173–190.
- Fanara JJ, Mensch J, Folguera G, Hasson E (2004) Developmental time and thorax length differences between the cactophilic species *Drosophila buzzatii* and *D. koepferae* reared in different natural hosts. *Evol Ecol* **18**:203–214.
- Hawthorne DJ, Via S (2001) Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature* **412**:904–907.
- Hosken DJ, Stockley P (2004) Sexual selection and genital evolution. *Trends Ecol Evol* **19**:8793.
- Hribar LJ (1996) Larval rearing temperature affects morphology of *Anopheles albimanus* (Diptera: Culicidae) male genitalia. *J Am Mosq Control Assoc* **12**:295–297.
- Iwata H, Ukai Y (2002) SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *J Hered* **93**:384–385.
- Kobayashi T (1998) Seasonal changes in body size and male genital structures of *Procladius choreus* (Diptera: Chironomidae: Tanyptodinae). *Aquat Insects* **20**:165–172.
- Kuhl FP, Giardina CR (1982) Elliptic Fourier features of a closed contour. *Computer Graphics and Image Processing*. **18**:236–258.
- Manfrin MH, Sene FM (2006) Cactophilic *Drosophila* in South America: a model for evolutionary studies. *Genetica* **126**:57–75.
- Manfrin MH, De Brito ROA, Sene FM (2001) Systematics and evolution of the *Drosophila buzzatii* (Diptera: Drosophilidae) cluster using mtDNA. *Ann Entomol Soc Am* **94**:333–346.
- Mayr E (1963) *Animal Species and Evolution*. Harvard University Press, Cambridge, MA.
- Monteiro SG, Sene FM (1995) Estudo morfométrico de populações de *Drosophila serido* das regiões Central e Sul do Brasil. *Rev Bras Genet* **18**:283.
- Pereira MAQR, Vilela CR, Sene FM (1983) Notes on breeding and feeding sites of some species of the repleta group of the genus *Drosophila* (Diptera, Drosophilidae). *Cien Cult* **35**:1313–1319.
- Rohlf FJ, Archie JW (1984) A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). *Syst Biol* **33**:302–317.
- Shapiro AM, Porter AH (1989) The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. *Annu Rev Entomol* **34**:231–245.
- Silva AFG, Sene FM (1991) Morphological geographic variability in *Drosophila serido* (Diptera, Drosophilidae). *Rev Bras Ent* **35**:455–468.
- Soto IM (2005) Use of elliptic Fourier descriptors for quantification of male genitalia morphology. *Dros Inf Serv* **88**:42–45.
- Soto IM, Carreira VP, Fanara JJ, Hasson E (2007a) Evolution of male genitalia: environmental and genetic factors affecting genital morphology in sibling *Drosophila* species and their hybrids. *BMC Evol Biol* **7**:77.
- Soto IM, Hasson E, Manfrin MH (2007b) Wing morphology is related to host plants in cactophilic *Drosophila gouveai* and *D. antonietae* (Diptera, Drosophilidae). *Biol J Linn Soc Lond* (in press).
- Soto IM, Manfrin MH, Sene FM, Hasson E (2007c) Viability and developmental time in the cactophilic *Drosophila gouveai* and *D. antonietae* (Diptera: Drosophilidae) are dependent of the cactus host. *Ann Entomol Soc Am* **4**:490–496.
- StatSoft, Inc. (2001) STATISTICA, Data Analysis Software System (version 6; available at <http://www.statsoft.com>).
- Stennett MD, Etges WJ (1997) Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. III. Epicuticular hydrocarbon variation is determined by use of different host plants in *Drosophila mojavensis* and *Drosophila arizonae*. *J Chem Ecol* **23**:2803–2824.
- Tidon-Sklorz R, Sene FM (2001) Two new species of the *Drosophila serido* sibling set (Diptera, Drosophilidae). *Iheringia Ser Zool* **90**:141–146.
- Vilela CR (1983) A revision of the *Drosophila repleta* species group (Diptera, Drosophilidae). *Rev Bras Entomol* **27**:1–114.
- Vilela CR, Sene FM (1977) Two new neotropical species of the repleta group of the genus *Drosophila* (Diptera, Drosophilidae). *Papeis Avulsos Zool* **30**:295–299.

Authors' addresses: Ignacio M. Soto, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria Pab. II, (C1428EHA) Buenos Aires, Argentina. E-mail: soto@ege.fcen.uba.ar; Helena Manfrin, Departamento de Biología, FFCLRP-USP, Av. dos Bandeirantes, 3900 Ribeirão Preto-SP, 14040-901, Brazil. E-mail: mhmanfri@rge.fmrp.usp.br; Esteban Hasson, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria Pab. II, (C1428EHA) Buenos Aires, Argentina. E-mail: ehasson@ege.fcen.uba.ar