Infection, Genetics and Evolution 19 (2013) 38–44

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Phenotypic plasticity in response to food source in *Triatoma infestans* (Klug, 1834) (Hemiptera, Reduviidae: Triatominae)

Julieta Nattero *, Romina Malerba, Claudia S. Rodríguez, Liliana Crocco

Cátedra de Introducción a la Biología, Facultad de Ciencias Exactas Físicas y Naturales, Instituto de Investigaciones Biológicas y Tecnológicas (IIByT) CONICET, Universidad Nacional de Córdoba, Avda. Vélez Sarsfield 299, piso 5, X5000JJC Córdoba, Argentina

ARTICLE INFO

Article history:
Received 21 March 2013
Received in revised form 10 June 2013
Accepted 13 June 2013
Available online 4 July 2013

Keywords:
Triatoma infestans
Phenotypic plasticity
Head morphometry
Food source
Full-sib family × environment interaction
Geometric morphometrics

ABSTRACT

In the Gran Chaco region of Argentina, Bolivia, and Paraguay, vector transmission of *Trypanosoma cruzi*, the etiological agent of Chagas disease, is still a severe problem because, among other causes, houses are reinfested with *Triatoma infestans*, the main vector of *T. cruzi* in southern South America. A better understanding of adaptation and evolution of *T. infestans* populations may contribute to the selection of appropriate vector control strategies in this region. Phenotypic plasticity is essential to understand development and maintenance of morphological variation. An experimental phenotypic plasticity study was conducted to assess if blood meal source induced head shape and size variation during development in *T. infestans*. Eighteen full-sib families were assigned to one of two food sources (pigeon and guinea pig) to examine the effect of food source on head shape and size in all nymph instars and adults. Data were analyzed using geometric morphometric tools and phenotypic plasticity analyses. Significant differences in head shape and size were observed between adults fed on different food sources. Allometric effects at the adult stage were observed. Head size showed significant food source × family interaction for fifth-instar nymphs and adults. For head size, significant differences between food sources were observed at stages and in ontogenetic trajectory. Phenotypic plasticity expression was found for head shape and size in adults; indeed, bugs fed on guinea pigs exhibited greater changes in head shape and larger heads than those fed on pigeon. Full-sib families exhibited different patterns of phenotypic expression in response to food source. Food source × family interaction may indicate that the observed variation in phenotypic plasticity may contribute to changes in head morphometry. These results may contribute to the selection of an appropriate control strategy for *T. infestans* in the Gran Chaco region, since they provide evidences of morphological plasticity in this species.

1. Introduction

Triatoma infestans (Hemiptera, Reduviidae: Triatominae) is the main vector of *Trypanosoma cruzi* in southern South America. The Southern Cone Initiative coordinated by the Pan American Health Organization drastically reduced the geographic range of *T. cruzi* and interrupted vector transmission in Uruguay, Chile, Brazil, and some areas of Argentina and Paraguay (Dias et al., 2002; Schmunis et al., 1996; Schofield et al., 2006). However, in the Gran Chaco region of Argentina, Bolivia, and Paraguay, the effectiveness of this programme was limited and vector transmission of *T. cruzi* still occurs (Gorla et al., 2009; Gürtler et al., 2007). One of the reasons for the persistence of *T. infestans* in the Chaco region is the reinfestation of houses after residual insecticide spraying (Cecere et al., 2006; Dujardin et al., 1997; Gürtler et al., 2004). A better understanding of different aspects of adaptation and evolution of *T. infestans* populations may contribute to the selection of appropriate vector control strategies in the Gran Chaco region.

Organisms often have flexibility in the expression of a character that helps them perform well in variable environments. This flexibility is called phenotypic plasticity and is considered essential for the understanding of the development and maintenance of variation in morphological size and shape (Pigliucci, 2005). The range of phenotypes might vary with different environmental conditions. As a result, morphological changes may be part of an adaptive response that depends on several factors ranging from physiological processes to environmental pressures (e.g., Ayala et al., 2011; Carreira et al., 2006; Thompson, 1999). Because evolution of morphological phenotypic plasticity entails genetic change in the environmental sensitivity of developmental trajectories (Falconer, 1990; Waddington, 1975), plastic morphological growth should be studied in the context of development (Pigliucci et al., 1996). Morphological traits associated with feeding (mandibles, head)

* Corresponding author. Tel.: +54 351 4332100x248; fax: +54 351 4332100.
E-mail addresses: jnattero@efn.uncor.edu (J. Nattero), rominamalerba@gmail.com (R. Malerba), claudiarodriguez@efn.uncor.edu (C.S. Rodríguez), lcrocco@efn.uncor.edu (L. Crocco).

1567-1348/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2013.06.014

can be related to food consumption (Pearson and Stemberger, 1980). This is particularly significant in haematophagous insect species, which find in food intake an important part of the resources used for reproduction (Bodin et al., 2009). Bugs belonging to the subfamily Triatominae are considered plastic insects that develop rapid morphological changes in response to adaptations to new habitats (Dujardin et al., 1999). Morphological variation, which has been frequently reported among conspecific populations of Triatominae, has been related to host preference and habitat (Dujardin et al., 2009), among other factors. *T. infestans* occurs mostly in domestic habitats, but is also present in peridomestic structures, such as chicken coops, store rooms, and goat corrals, taking blood both from domestic bird and mammal hosts. Evidences of morphological variation in response to different food sources have been reported for this species. Accordingly, Schachter-Brodie et al. (2004) found differences in the morphology of wings in relation to host association. Abrahan et al. (2008) reported heterogeneity of the antennal phenotypes of closely related populations living in goat corrals, rabbit cages and intradomestic environments, whereas Hernández et al. (2011) found population structure in head and wing morphometry in individuals collected from goat corrals and chicken coops. Since feeding source is known to affect different aspects of nymphal development and reproduction in *T. infestans* (Guarneri et al., 2000; Nattero et al., 2011), blood meal source is expected to have a direct influence on different phenotypic dimensions in the ontogenetic trajectory. Food sources would represent environments that are different enough to influence development, causing significant variations in head shape and size (e.g., Esperk and Tammaru, 2010; Jorge et al., 2011; Laparie et al., 2010). The ecological characteristics and the current knowledge of *T. infestans* make this species appropriate for studying diet-induced phenotypic plasticity. Hence, the aim of the present study was to investigate the influence of blood meal source on the development of *T. infestans*, with a focus on variation in head size and shape, which was decomposed by using geometric morphometric tools and phenotypic plasticity analysis. All stages of ontogeny were monitored and measurements of head size and shape were used to identify diet-induced phenotypic plasticity. Specifically, different blood meal sources were explored as potential causes of differences in head shape and size among nymph instars, females and males. In addition, the variation among full-sib families was quantified and the interaction of full-sib family with food source and stage was measured to determine the magnitude and pattern of expression of genetic variation for phenotypic plasticity during ontogeny.

2. Methods

2.1. Insects

First laboratory generation insects of a *T. infestans* population were used; they were collected from peridomestic structures (chicken coops) from Belgrano, San Luis province, Argentina. Field-collected bugs were maintained in the laboratory and fed regularly on pigeon (*Columba livia*) until molting to adult stage. One male and one female from these newly emerged adults were held in cylindrical glass vials; the entire offspring of each couple was considered a full-sib family. First-instar nymphs from the 18 full-sib families were haphazardly assigned to one of the two food sources used: guinea pigs (*Cavia porcellus*) or pigeons. For all assays, seven pigeons and six guinea pigs were used. For bug feeding, pigeons were immobilized and guinea pigs were placed in small plastic cases, following the ethics guidelines for biomedical research from our institution, based on resolution No. 1047 (2005) of the National Council of Scientific and Technical Research (CONICET). For each assay, up to five bugs were used per host, which were allowed to feed *ad libitum* until the bug itself removed its proboscis without trying to probe again. All individuals were fed regularly (every 15 days) on the same food source, but not necessarily on the same animal, during the entire life cycle. During all the experiments, insects were maintained in the laboratory at 26 ± 2 °C, 60–70% relative humidity and a photoperiod of 12:12 h (light: dark). Four to five individuals of each stage (nymphs from first to fifth instars, females and males) per full-sib family were photographed in lateral view with a Nikon D200 camera. A total of 1024 individuals, including all the stages, were photographed. Adults were also photographed in frontal view.

2.2. Data acquisition and morphometric analysis

We measured the total length of the adult insects from the clypeus to the abdominal tip using the frontal view photographs. Measurements were taken in UTHSCSA Image Tool (version 3.0 for Windows, San Antonio, TX, USA).

For the geometric morphometric analysis, head shape descriptors using landmark-based methodology were recorded. Landmarks should represent homologous anatomical loci, providing adequate coverage of the overall morphology, and should be found repeatedly and reliably (Zelditch et al., 2004). Six coplanar landmarks located along the outline of head were defined and collected using TPSDig, version 2.1 (Rohlf, 2006). Landmarks were collected only from the right side of heads to avoid interference in the analyses of within-individual variation (Fig 1a). To explore possible differences in head shape induced by the food sources at different nymph stages, we performed a Hotelling's T^2 test to compare head shape among full-sib families fed on each food source (Zelditch et al., 2004). For this purpose, a consensus head shape per full-sib family (Rohlf, 2006) was first obtained by performing a general Procrustes analysis in TPSRelw, version 1.49. This analysis removed non-shape variation (i.e., translation, scaling and rotation) in the landmark coordinates (Zelditch et al., 2004). The shape variables calculated, called partial warps, indicate partial contributions of hierarchically scaled vectors spanning a linear shape space. The matrix of partial warp scores was complemented by two uniform dimensions of shape change.

For adult individuals, a Hotelling's T^2 was performed to test for the existence of differences in shape between sexes. Otherwise, sexual dimorphism could mask variations induced by food source. Additionally, a head shape consensus for females and males for each feeding source was obtained using TPSRelw, version 1.49 (Rohlf, 2006). To visualize the displacement of landmarks relative to a theoretical consensus for each group (females and males per feeding source), the thin-plate spline procedure was applied using TPSSplin, version 1.49 (Rohlf, 2006). This procedure smoothes configuration by minimizing the 'bending energy' of deformation (see Zelditch et al., 2004). To describe differences in head shape among groups, a canonical variate analysis (CVA) on the partial warp scores was performed. Pairwise multiple comparisons based on the generalized Mahalanobis distance ($D2$) from CVA were performed to determine the group that differed statistically in head shape. The analysis was performed using PAST (Hammer et al., 2001).

To investigate trends in shape change, the dimensionality of the matrix of partial warps and uniform component scores was further reduced by relative warps (RWs) analysis (Bookstein, 1991), a principal component analysis (PCA) of the partial warp and uniform components. Relative warps of each individual were used to investigate allometric and phenotypic plasticity analysis on head shape. Calculation of RWs was performed using TpsRelw version 1.49 (Rohlf, 2006). To investigate allometric occurrence on females and males for the two food sources, we performed a multivariate

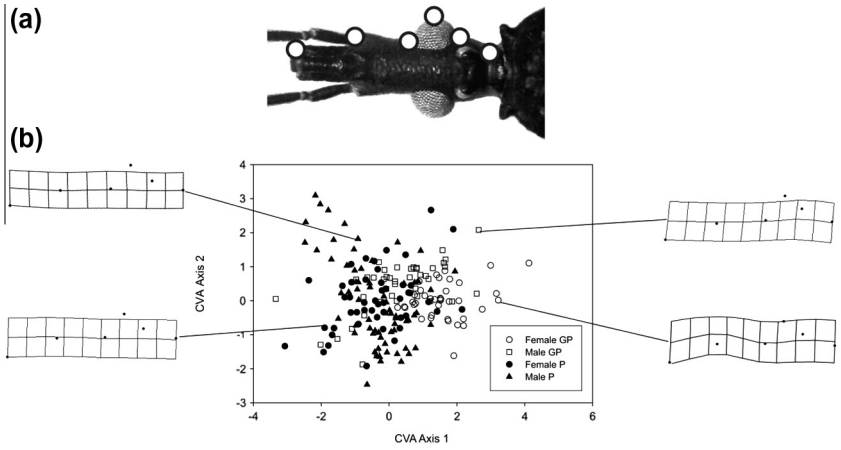

Fig. 1. Geometric morphometric analysis in adults of *Triatoma infestans*. (a) Head in lateral view showing the six landmarks located along the outline of the head. (b) Ordination of the groups (females and males fed on guinea pigs and pigeons) along the first two axes in CVA together with deformation grids showing the head shape deformation for each group (females and males fed on guinea pigs and pigeons). GP: guinea pig, P: pigeon.

analysis of covariance (MANCOVA) of the RWs, considering food source as the categorical predictor and centroid size as the covariate. This procedure allowed us to test simultaneously for the effect of size on shape (allometric effect), for the effect of food source on shape variation, independent of size, i.e., the nonallometric component, and for the consistency of the allometric effect between food sources (size *x* food source interaction) (Debat et al. 2003).

We also conducted a multivariate regression of the RWs on centroid size both for females and males. The residual values were then used as shape variables independent of size (the nonallometric component) for the phenotypic plasticity analysis (see below).

2.3. Phenotypic plasticity analysis

Phenotypic plasticity represents measureable morphometric variation, and as such it can often be expressed and analyzed with analysis of variance (Pigliucci, 2001).

To test phenotypic plasticity on head shape variables independent of size (the nonallometric component), the residual values of multivariate regression of the RWs on centroid size both for females and males were used as dependent variables in a MANOVA. This analysis was used to investigate the relative importance of (1) food source, (2) full-sib family (presence of average phenotypic plasticity regardless of families) and (3) the food source × family interaction term as main sources of variation (variation of plasticity among families suggesting a cost in phenotypic plasticity) in explaining head shape across the first to fifth nymph instars and adults.

Centroid size (CS) of each individual was used to conduct the phenotypic plasticity analysis on head size. CS is a single variable of size that integrates different axes of growth and is measured as the square root of the sum of the squared distances between the center of the configuration of landmarks and each individual landmark (Bookstein, 1991). CS was obtained using MOG module from CLIC package. For this purpose, landmarks collected with TPSdig were converted with TET module from CLIC package into a format that is readable by MOG software. The assumption of normality for CS included in the analysis was tested using the Shapiro–Wilks test. To determine whether CS varied between food sources (guinea pig and pigeon), a *T*-test for independent samples was performed.

For head size variation, a full mixed-model ANOVA was conducted to investigate the relative importance of full-sib family, food source, and food source × family interaction as main sources of variation across the first to fifth nymph instars and adults. Food source was considered fixed, family and its interactions were considered random. The estimation of CS was useful to understand the relationship between size and shape of heads. All ANOVA and MANOVA assumptions were properly checked.

To perform a detailed analysis of all stages (nymph instars and adults) throughout the full-sib family, and the interaction between stage and family and stage and food source (nutrient effect) in relation to size variation, an ANCOVA model was used. The model included: y = stage (covariate), full-sib family, food source, full-sib family × food source, stage × full-sib family, stage × food source, where the interaction terms involving stage were included to investigate the variation of stage at the family level (i.e., stage - × full-sib family term) or at the food source level (i.e. stage × food source term). The family × food source interaction term investigates the plasticity of ontogenetic trajectories. The three-way interaction was excluded because it was not significant in any case. We decided to use an ANCOVA model instead of a repeated measure ANOVA based on the independence of the trait expression at each stage of the family (e.g., Pigliucci and Schlichting, 1995; Pigliucci, 1997).

3. Results

3.1. Morphometric analysis

Adults fed on pigeons showed larger body size than those fed on guinea pigs, both for females and males (pigeons: 27.11 ± 0.14 and 25.33 ± 0.15 mm for females and males respectively, guinea pigs: 25.79 ± 0.18 and 24.60 ± 0.19 mm for females and males respectively, females $F_{(143.1)} = 30.18$, $p < 0.0001$; males $F_{(147.1)} = 12.95$, $p < 0.001$).

Results from the Hotelling's T^2 tests showed that females significantly differed from males in head shape variation, regardless of feeding source (T^2: 12.44, $F = 2.36$, p: 0.049; T^2: 12.35, $F = 2.38$, p: 0.04249 for guinea pig and pigeon, respectively). For each nymph instar, Hotelling's T^2 tests did not show significant differences between feeding sources (data not shown). CVA revealed overall and pairwise differences in head shape for all comparison of females and males from both feeding sources (Wilk's $\lambda = 0.916$, F: 5.829, $p < 0.025$; Mahalanobis distance, $p < 0.05$ for the six contrasts, Fig. 1b). Deformation grids revealed that head shape deformations were greater for bugs, both females and males, fed on guinea pigs than for those fed on pigeons (Fig 1b).

Table 1
MANOVA testing for head shape differences in the five nymph instars, females and males in *Triatoma infestans* fed on two different sources (guinea pig and pigeon).

Head shape	*n*	MANOVA Wilk's value	Full-sib family		Food source		Food source × family	
		Whole model	*F*	*p* value	*F*	*p* value	*F*	*p* value
First instar	150	0.232	0.059	0.136	0.009	0.260	0.287	0.287
Second instar	144	0.321	0.087	0.101	0.090	0.101	0.029	0.201
Third instar	149	0.128	0.008	0.543	0.006	0.611	0.014	0.295
Fourth instar	144	0.401	0.032	0.245	0.052	0.112	0.023	0.194
Fifth instar	145	0.732	0.063	0.125	0.227	0.000	0.011	0.213
Females	144	0.622	0.185	0.036	0.225	0.000	0.112	0.103
Males	148	0.575	0.210	0.023	0.398	0.000	0.080	0.087

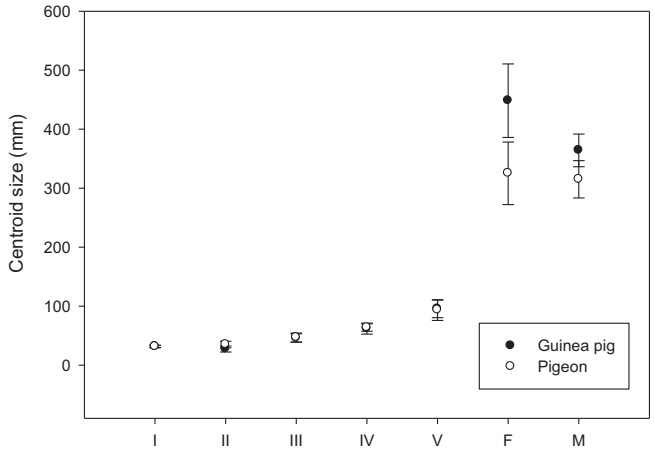

Fig. 2. Head size variation among the five nymph instars and adults of *Triatoma infestans* fed on two food sources. I: first-instar nymphs, II: second-instar nymphs, III: third-instar nymphs, IV: fourth-instar nymphs, V: fifth-instar nymphs, F: females, M: males.

Results from the MANCOVAs to test for allometric occurrence showed that food source and size effects were found to be significant for females and males (Females: Wilk's $\lambda = 0.389$, $F_{7.136} = 1.267$, $p = 0.029$; Wilk's $\lambda = 0.79$, $F_{7.136} = 2.89$, $p < 0.01$ for food source and size effects respectively; Males: Wilk's $\lambda = 0.415$, $F_{7.140} = 1.02$, $p = 0.043$; Wilk's $\lambda = 0.74$, $F_{7.140} = 1.25$, $p < 0.01$ for food source and size effects, respectively), suggesting both an effect of food source on the nonallometric component of shape and an allometric effect. The interaction term (size *x* food source) was non-significant both for females and males (Wilk's $\lambda = 0.035$, $F_{7.136} = 0.01$, ns; Wilk's $\lambda = 0.024$, $F_{7.136} = 0.05$, ns, respectively) indicating that the allometric effect remains relatively constant between the two food sources. The multivariate regression of RWs on centroid size confirmed the occurrence of a strong allometric effect, since there were significant contributions of centroid size to shape variation both in females and males fed on guinea pigs and pigeons ($p < 0.01$ in all cases).

3.2. Phenotypic plasticity analysis

MANOVA for head shape variables independent of size (the nonallometric component) showed that for nymphs from first to fourth instars, no effect of food source on head shape was evident (Table 1). Nevertheless, fifth-instar nymphs, females and males exhibited significant differences in head shape for food source variation (Table 1). At the family level, significant effects of food source on head shape was shown for females and males (Table 1). No significant food source × family interaction was observed (Table 1).

CS variation between food sources for each stage is shown in Fig. 2. Results from the *T*-test for independent samples to compare CS between food sources at each stage showed that only adults exhibited significant differences in head size expression, being larger for females than for males for both food sources ($T = 85.39$, $p < 0.0001$; $T = 17.75$, $p < 0.0001$ for females and males, respectively) (Fig. 2). In addition, CS was larger for bugs fed on guinea pigs than for those fed on pigeons (Fig. 2).

No effect of food source on head size of *T. infestans* nymphs was evident in the first ANOVA model (Table 2). Nevertheless, both females and males exhibited significant differences in head size expression for both food sources (Table 2). At the family level, no significant effects of food source on head size was shown by the first ANOVA model (Table 2). A significant food source × family interaction was observed for fifth-instar nymphs, females and males, showing variation in plasticity among families and suggesting a cost in phenotypic plasticity at these stages (Table 2, Fig. 3). The ANCOVA model showed a significant stage term (Table 3). The interaction terms stage × food source and family × food source were significant (Table 3).

4. Discussion

This study provides three main results related to diet- induced phenotypic plasticity and full-sib family variation in phenotypic plasticity of *T. infestans*: head shape phenotypic plasticity, head size phenotypic plasticity and full-sib family variation in head size. Our study depicted that morphological changes in head, both in

Table 2
ANOVA for head size for the five nymph instars, females and males in *Triatoma infestans* fed on two different sources (guinea pig and pigeon). The full-sib family term and its interactions are random.

Head centroid size	*n*	Full-sib family		Food source		Food source × family	
		MS	*p* value	MS	*p* value	MS	*p* value
First instar	150	8.553	$p = 0.141$	2.045	$p = 0.470$	8.970	$p = 0.132$
Second instar	144	9.997	$p = 0.559$	9.171	$p = 0.576$	53.095	$p = 0.181$
Third instar	149	61.571	$p = 0.292$	21.491	$p = 0.533$	38.596	$p = 0.404$
Fourth instar	144	2.217	$p = 0.854$	59.109	$p = 0.344$	12.254	$p = 0.665$
Fifth instar	145	6.938	$p = 0.859$	6.350	$p = 0.857$	3456.320	$p = 0.000$
Females	144	1171.16	$p = 0.620$	357101	$p = 0.000$	23442.5	$p = 0.023$
Males	148	88.120	$p = 0.765$	17182.6	$p = 0.000$	5168.53	$p = 0.057$

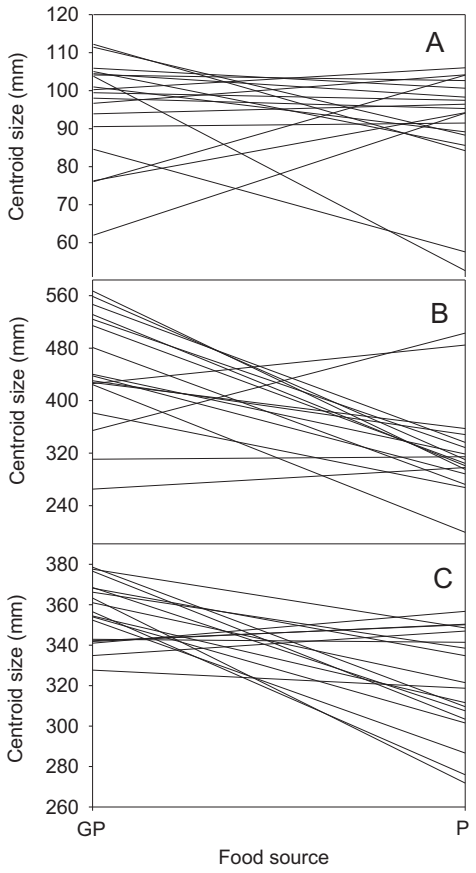

Fig. 3. Full-sib family variation for food source phenotypic plasticity of head size for 18 full-sib families in fifth-instar nymphs (A), females (B) and males (C) of *Triatoma infestans*. Each line connects the mean head centroid size for each full-sib family fed on guinea pigs (GP) and pigeons (P).

size and shape, may occur within a single generation. Characters able to change as fast as the environment does may be called "labile" characters (Scheiner, 1993), and are considered important in phenotypic evolution (West-Eberhard, 1989). Head shape variation among *T. infestans* individuals fed on two food sources showed significant differences both for females and males. Additionally, the nonallometric component of shape variation used for the phenotypic plasticity analysis showed that at the full-sib family level, significant effects of food source on head shape was detected for adults. Although size variation in Triatominae species related to host has been attributed to nutritional quality in a few cases (Guarneri et al., 2000; Hernández et al., 2011), this correlation is not straightforward for shape variation. Unlike size, there are very few evidences in triatomines that the shape of a given structure is influenced by the quality of blood ingested (but see Hernández et al., 2011; Schachter-Brodie et al., 2004).

Triatoma infestans showed evidences of phenotypic plasticity in head size only at the adult stage. Plastic responses to different quantity or quality of the diet have been reported for other insect species (e.g., Esperk and Tammaru, 2010; Jorge et al., 2011; Laparie et al., 2010). The present results showed that females exhibited significantly greater head CS than males for both food sources. The greater head size of *T. infestans* adults fed on guinea pigs is not consistent with variation of body size, since our results showed that both females and males fed on pigeons showed larger body size than those fed on guinea pigs. Although body size seems to respond to food source, phenotypic plasticity in head size was not a simple consequence of allometric plasticity between body and head size. The diet-induced phenotypic plasticity in head size for bugs fed on guinea pigs seems to be due to changes in developmental allocation to tissue growth that maintain growth of head size while reducing growth of body size. The reduced growth in body size was probably due to the reduced amount of blood ingested, as shown for *T. infestans* and for other triatominae species fed on guinea pigs relative to those fed on pigeon (e.g., Guarneri et al., 2000; Martínez-Ibarra et al., 2003; Sant'Anna et al., 2001).

Our results suggest that differences observed in head shape and size of adults fed on two different food sources could be due to a plastic response to the type of blood they are ingesting. The head capsules of bugs contain cibarial pumps; their movements are regulated by a complex of muscles that transfer the blood from the host to the bug gut during feeding. To do this, the cibarial pumps create a negative pressure difference between tip of mouthpart and muscular cavitation of the pump lumen (Lehane, 1991). The pump and its associated muscle nearly fill the head capsule in *Rhodnius prolixus*, another triatomine species (Smith, 1979; Smith and Friend, 1970). In triatominae, efficient antihaemostatic activity in response to haemostasis of a particular host would facilitate the maintenance of a steady flow of blood during feeding. Birds and mammals exhibited differences in the haemostatic mechanism. Although the basic mechanisms of haemostasis are conserved among vertebrates, there are marked differences among the different classes (Lewis, 1996). For example, the intrinsic coagulation cascade pathway is a very important haemostatic mechanism in mammals but has a much less important role in birds. In addition, the thrombocytes of birds, which perform a function similar to that of platelets in mammals, are less efficient and do not respond to ADP, an important inducer of platelet aggregation (Lewis, 1996). Differences in haemostasis of the hosts and the antihaemostatic activity of triatomine saliva could modulate the insect feeding process. On the other hand, viscosity of the diet is known to interfere with feeding rate (Lehane, 1991), and is high in mammals compared to birds (Windberger and Baskurt, 2007). Considering that muscle nearly fill head capsule, that viscosity of the diet interferes with the feeding rate and that haemostasis mechanism is different between birds and mammals, the changes observed in head size and shape through phenotypic plasticity analysis could be due to an adaptation of *T. infestans* to its habitual hosts and hence a further development of the muscles of the cibarial pump in individuals fed on guinea pig. In other experimental studies in Triatominae, individuals fed on blood of different nutritional quality showed differences in body size, reproductive parameters and/or life cycle characteristics (e.g., Guarneri et al., 2000; Martínez-Ibarra et al., 2003; Nattero et al., 2011; Sant'Anna et al., 2001). Besides the overall body size, in other insects such as grasshoppers and butterflies, shape and size of head or trophic appendages were found to vary

Table 3
Result of ANCOVA for morphometry of heads in *Triatoma infestans* fed on two different sources (guinea pig and pigeon). The full-sib family term is random. Stage (five nymph instars, females and males) is the covariate.

Head centroid size	Stage	Full-sib family	Food source	Stage × family	Stage × food source	Food source × family
	$F_{5.904}$ = 1827.42 p = 0.000	$F_{17.904}$ = 7.652 p = 0.924	$F_{1.904}$ = 7116 p = 0.000	$F_{85.904}$ = 0.090 p = 0.997	$F_{5.904}$ = 58.512 p = 0.001	$F_{17.904}$ = 7.477 p = 0.007

among juvenile stages of individuals fed on different diets in only one generation (Ohata et al., 2011; Thompson, 1992, 2001).

Phenotypic variation is partitioned between genetic (full-sib family) and environmental effects. The interaction between full-sib family and environment represents genetic variation for phenotypic plasticity. The present results suggest a full-sib family-based difference in the expression of phenotypic plasticity for head size. Full-sib family variation for phenotypic plasticity changed through ontogeny and during the ontogenetic trajectory. Full-sib family variation for phenotypic plasticity of head size was evident in the fifth-instar nymph and adults. In early ontogeny, from first to fourth instars, before plastic morphological responses to the feeding environment are expressed, full-sib reaction norms might have been flat and parallel (Thompson, 2001). In subsequent stages of ontogeny, full-sib families varied in the timing of their developmental responses to food source; indeed, some families exhibited plastic responses while others remained invariant across environments, thereby generating family × food source interaction. In other words, different full-sib families exhibited a different pattern of phenotypic expression across a range of environments, in this particular study, food source. This result may indicate that the observed variation in the expression of phenotypic plasticity may contribute to evolutionary changes in head morphometry (West-Eberhard, 2003), which deserves further research. Other studies on diet-induced differences in body size or responses to artificial selection of environmental sensitivity have detected genetic variation for plasticity of body size (Falconer, 1990; Hillesheim and Stearns, 1991; Merckx et al., 2006; Nielsen and Andersen, 1987; Thompson, 2001).

Triatominae are considered plastic insects that develop rapid morphological changes in response to environmental variability (Dujardin et al., 2009). This agrees with the assumption that species with high levels of plasticity in morphological and life-history traits have a great capacity to deal with changing and highly variable environments. Most of the studies on different aspects of the blood-sucking bug *T. infestans* showed evidence of the high potential of this species as vector of Chagas disease (e.g., Asin and Crocco de Ayerbe, 1992; Catalá de Montenegro, 1989; Guarneri et al., 2000; Zeledón, 1983). However, to our knowledge, this is the first study that examined if the quality of blood ingested during nymph instars and adult stages induces phenotypic plasticity in head morphometry and if there are full-sib family-based differences in the expression of phenotypic plasticity in this species. Our results indicate that although there is no direct effect of diet-induced head shape variation and phenotypic plasticity on head size in nymphs, there are evidences of diet-induced phenotypic plasticity in head shape and size in adults, both in females and males. Moreover, bugs fed on guinea pigs exhibited higher head shape changes and larger head than those fed on pigeon. Evidences of full-sib family variation for phenotypic plasticity were also observed in head size at the fifth-instar and adult stages, suggesting that the basis for expression of phenotypic plasticity is important in determining full-sib family variability, depending on the food source. Studies on phenotypic plasticity in Triatominae should receive more attention as they might contribute to the understanding of the dynamics that underlie the evolution of morphological plasticity in this subfamily.

Acknowledgments

We are grateful to G. Muscicant and C. Cano (Servicio Provincial de Chagas San Luis) for logistic support in fieldwork. CR and JN are scholarship holder and researcher at CONICET, respectively. This study was funded by Secretaría de Ciencia y Técnica Universidad Nacional de Córdoba (05/1554).

References

Abrahan, L., Hernández, L., Gorla, D., Catalá, S., 2008. Phenotypic diversity of *Triatoma infestans* at the microgeographic level on the Gran Chaco of Argentina and the Andean valleys of Bolivia. J. Med. Entomol. 45, 660–666.
Asin, S., Crocco de Ayerbe, L.B., 1992. Influence of mating on ovarian follicle development in *Triatoma infestans* (Klug, 1834). Mem. Inst. Oswaldo Cruz 87, 369–374.
Ayala, D., Caro-Riaño, H., Dujardin, J.-P., Rahola, N., Simard, F., Fontenille, D., 2011. Chromosomal and environmental determinants of morphometric variation in natural populations of the malaria vector *Anopheles funestus* in Cameroon. Infect. Genet. Evol. 11, 940–947.
Bodin, A., Vinauger, C., Lazzari, C.R., 2009. Behavioural and physiological state dependency of host seeking in the blood-sucking insect *Rhodnius prolixus*. J. Exp. Biol. 212, 2386–2393.
Bookstein, F.L., 1991. Morphometric tools for landmark data. Cambridge University Press, Cambridge.
Carreira, V.P., Soto, I.M., Hasson, E., Fanara, J.J., 2006. Patterns of variation in wing morphology in the cactophilic *Drosophila buzzatii* and its sibling *D. koepferae*. J. Evol. Biol. 19, 1275–1282.
Catalá de Montenegro, S.S., 1989. Relaciones entre consumo de sangre y ovogénesis en *Triatoma infestans* Klug 1934 (Hemiptera: Reduviidae). Chagas 5, 3–10.
Cecere, M.C., Vazquez-Prokopec, G.M., Gürtler, R.E., Kitron, U., 2006. Reinfestation sources for Chagas disease vector, *Triatoma infestans*, Argentina. Emerg. Inf. Dis. 12, 1096–1102.
Debat, V., Begin, M., Legout, H., David, J.R., 2003. Allometric and nonallometric components of Drosophila wing shape respond differently to developmental temperature. Evolution 57, 2773–2784.
Dias, J.C.P., Silveira, A.C., Schofield, C.J., 2002. The impact of Chagas disease control in Latin America. A review. Mem. Inst. Oswaldo Cruz 97, 603–612.
Dujardin, J.P., Bermúdez, H., Schofield, C.J., 1997. The use of morphometrics in entomological surveillance of sylvatic foci of *Triatoma infestans* in Bolivia. Acta Trop. 66, 145–153.
Dujardin, J.P., Panzera, F., Schofield, C., 1999. Triatominae as a model of morphological plasticity under ecological pressure. Mem. Inst. Oswaldo Cruz 94, 223–228.
Dujardin, J.P., Costa, J., Bustamante, D., Jaramillo, N., Catalá, S.S., 2009. Deciphering morphology in Triatominae: the evolutionary signals. Acta Trop. 110, 101–111.
Esperk, T., Tammaru, T., 2010. Size compensation in moth larvae: attention to larval instars. Physiol. Entomol. 35, 222–230.
Falconer, D.S., 1990. Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance. Genet. Res. 56, 57–70.
Gorla, D.E., Porcasi, X., Hrellac, H., Catalá, S., 2009. Spatial stratification of house infestation by *Triatoma infestans* in La Rioja, Argentina. Am. J. Trop. Med. Hyg. 80, 405–409.
Guarneri, A., Pereira, M., Diotaiuti, L., 2000. Influence of the blood meal source on the development of *Triatoma infestans*, *Triatoma brasiliensis*, *Triatoma sordida*, and *Triatoma pseudomaculata* (Heteroptera, Reduviidae). J. Med. Entomol. 37, 373–379.
Gürtler, R.E., Canale, D.M., Spillman, C., Stariolo, R., Salomon, D.O., Blanco, S., 2004. Effectiveness of residual spraying of peridomestic ecotopes with deltamethrin and permethrin on *Triatoma infestans* in rural western Argentina: a district-wide randomized trial. Bull WHO 82, 196–205.
Gürtler, R.E., Kitron, U., Cecere, M.C., Segura, E.L., Cohen, J.E., 2007. Sustainable vector control and management of Chagas disease in the Gran Chaco, Argentina. Proc. Natl. Acad. Sci. USA 104, 16194–16199.
Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. University of Oslo, Oslo.
Hernández, M.L., Abrahan, L.B., Dujardin, J.P., Gorla, D.E., Catalá, S.S., 2011. Phenotypic variability and population structure of peridomestic *Triatoma infestans* in rural areas of the arid Chaco (Western Argentina): spatial influence of macro- and microhabitats. Vector Borne Zoonotic 11, 503–513.
Hillesheim, E., Stearns, S.C., 1991. The responses of *Drosophila melanogaster* to artificial selection on body weight and its phenotypic plasticity in two larval food environments. Evolution 45, 1909–1923.
Jorge, L.R., Cordeiro-Estrela, P., Klaczko, L.B., Moreira, G.R.P., Freitas, A.V.L., 2011. Host-plant dependent wing phenotypic variation in the neotropical butterfly *Heliconius erato*. Biol. J. Linn. Soc. 102, 765–774.
Laparie, M., Lebouvier, M., Lalouette, L., Renault, D., 2010. Variation of morphometric traits in populations of an invasive carabid predator (*Merizodus soledadinus*) within a sub-Antarctic island. Biol. Invasions 12, 3405–3417.
Lehane, M.J., 1991. Biology of Blood-sucking Insects. Harper-Collins Academic, London.
Lewis, J.H., 1996. Comparative Haemostasis in Vertebrates. Plenum Press, New York.
Martínez-Ibarra, J.A., Novelo López, N., Hernández Robles, M.R., Grant Guillén, J., 2003. Influence of the blood meal source on the biology of Meccus picturatus Usinger 1939 (Hemiptera: Reduviidae: Triatominae) under laboratory conditions. Mem. Inst. Oswaldo Cruz 98, 227–232.
Merckx, T., Karlsson, B., Van Dyck, H., 2006. Sex- and landscape-related differences in flight ability under suboptimal temperatures in a woodland butterfly. Funct. Ecol. 20, 436–441.
Nattero, J., Rodríguez, C., Crocco, L., 2011. Influence of the quality and quantity of blood ingested on reproductive parameters and life-span in *Triatoma infestans* (Klug). Acta Trop. 97, 183–187.

Nielsen, V.H., Andersen, S., 1987. Selection for growth on normal and reduced protein diets in mice. Genet. Res. 50, 7–15.
Ohata, M., Furumoto, A., Ohsaki, N., 2011. Plastic changes in head size during juvenile development of the butterfly *Pieris napi*. Ecol. Res. 26, 541–546.
Pearson, D.L., Stemberger, S., 1980. Competition, body size and the relative energy balance of adult tiger beetles (Coleoptera: Cicindelidae). Am. Midl. Nat. 104, 373–377.
Pigliucci, M., 1997. Ontogenetic phenotypic plasticity during the reproductive phase in *Arabidopsis thaliana* (Brassicaceae). Am. J. Bot. 84, 887–895.
Pigliucci, M., 2001. Phenotypic Plasticity: Beyond Nature and Nurture. Johns Hopkins University Press, Baltimore.
Pigliucci, M., 2005. Evolution of phenotypic plasticity: where are we going now? Trends Ecol. Evol. 20, 481–486.
Pigliucci, M., Schlichting, C.D., 1995. Ontogenetic reaction norms in *Lobelia siphilitica* (Lobeliaceae): response to shading. Ecology 76, 2134–2144.
Pigliucci, M., Schlichting, C., Jones, C., Schwenk, K., 1996. Developmental reaction norms: the interactions among allometry, ontogeny, and plasticity. Plant Spec. Biol. 11, 69–85.
Rohlf, F.J., 2006. TPSDig 2.10. Department of Ecology and Evolution, State University of New York at Stony Brook. Stony Brook, New York.
Sant'Anna, M.R., Diotaiuti, L., de Figueiredo Gontijo, A., de Figueiredo Gontijo, N., Pereira, M.H., 2001. Feeding behaviour of morphologically similar Rhodnius species: influence of mechanical characteristics and salivary function. J. Insect Physiol. 47, 1459–1465.
Schachter-Brodie, J., Dujardin, J.-P., Kitron, U., Gürtler, R., 2004. Spatial structuring of *Triatoma infestans* (Hemiptera, Reduviidae) populations from Northwestern Argentina using wing geometric morphometry. J. Med. Entomol. 41, 643–649.
Scheiner, S.M., 1993. Genetics and evolution of phenotypic plasticity. Annu. Rev. Ecol. Syst. 24, 35–68.
Schmunis, G., Zicker, F., Moncayo, A., 1996. Interruption of Chagas disease transmission through vector elimination. Lancet 348, 1171.
Schofield, C.J., Jannin, J., Salvatella, R., 2006. The future of Chagas disease control. Trends Parasitol. 22, 583–588.
Smith, J.J.B., 1979. Effect of diet viscosity on the operation of the pharyngeal pump in the blood-feeding bug *Rhodnius prolixus*. J. Exp. Biol. 82, 93–104.
Smith, J.J.B., Friend, W.G., 1970. Feeding in *Rhodnius prolixus*: responses to artificial diets as revealed by changes in electrical resistance. J. Insect Physiol. 19, 1709–1720.
Thompson, D.B., 1992. Consumption rates and the evolution of diet-induced plasticity in the head morphology of *Melanoplus femurrubrum* (Orthoptera. Acrididae). Oecologia 89, 204–213.
Thompson, D.B., 1999. Different spatial scales of natural selection and gene flow: the evolution of behavioral geographic variation and phenotypic plasticity. In: Foster, S., Endler, J. (Eds.), Geographic Diversification of Behavior: An Evolutionary Perspective. Oxford University Press, Oxford, pp. 33–51.
Thompson, D.B., 2001. Genotype–environment interaction and the ontogeny of diet-induced phenotypic plasticity in size and shape of *Melanoplus femurrubrum* (Orthoptera: Acrididae). J. Evol. Biol. 12, 38–48.
Waddington, C.H., 1975. The Evolution of an Evolutionist. Cornell University of Press, New York.
West-Eberhard, M., 1989. Phenotypic plasticity and the origins of diversity. Annu. Rev. Ecol. Syst. 20, 249–278.
West-Eberhard, M.J., 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.
Windberger U. and Baskurt O.K. 2007. Comparative hemorheology, In: Baskurt O.K., Hardeman M.R., Rampling M.W. and Meiselma H.J., (Eds.), *Handbook of Hemorheology and Hemodynamics*, 2007, IOS Press; Amsterdan, 267-288.
Zelditch, M.L., Swiderski, D.L., Sheets, D.H., Fink, W.L., 2004. Geometric Morphometrics for Biologists: A Primer. Elsevier Academic Press, New York.
Zeledón, R., 1983. Vectores de la Enfermedad de Chagas y sus características ecofisiológicas. Interciencia 8, 384–395.