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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

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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).

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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