

Host-feeding sources and habitats jointly affect wing developmental stability depending on sex in the major Chagas disease vector *Triatoma infestans*



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ARTICLE INFO

Article history:

Received 1 June 2015

Received in revised form 24 August 2015

Accepted 25 August 2015

Available online 28 August 2015

Keywords:

Fluctuating asymmetry

Environmental variance

Geometric morphometrics

Triatominae

Wing morphology

ABSTRACT

Fluctuating asymmetry (FA), a slight and random departure from bilateral symmetry that is normally distributed around a 0 mean, has been widely used to infer developmental instability. We investigated whether habitats (ecotopes) and host-feeding sources influenced wing FA of the hematophagous bug *Triatoma infestans*. Because bug populations occupying distinct habitats differed substantially and consistently in various aspects such as feeding rates, engorgement status and the proportion of gravid females, we predicted that bugs from more open peridomestic habitats (i.e., goat corrals) were more likely to exhibit higher FA than bugs from domiciles. We examined patterns of asymmetry and the amount of wing size and shape FA in 196 adult *T. infestans* collected across a gradient of habitat suitability and stability that decreased from domiciles, storerooms, kitchens, chicken coops, pig corrals, to goat corrals in a well-defined area of Figueroa, northwestern Argentina. The bugs had unmixed blood meals on human, chicken, pig and goat depending on the bug collection ecotope. We documented the occurrence of FA in wing shape for bugs fed on all host-feeding sources and in all ecotopes except for females from domiciles or fed on humans. FA indices for wing shape differed significantly among host-feeding sources, ecotopes and sexes. The patterns of wing asymmetry in females from domiciles and from goat corrals were significantly different; differences in male FA were congruent with evidence showing that they had higher mobility than females across habitats. The host-feeding sources and habitats of *T. infestans* affected wing developmental stability depending on sex.

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1. Introduction

Triatoma infestans (Hemiptera, Reduviidae, Triatominae) is the epidemiologically most important vector of Chagas disease (caused by *Trypanosoma cruzi*) in southern South America. The main habitats of *T. infestans* in rural houses are human-made or -modified structures used as sleeping quarters or for housing domestic animals on which they blood-feed (Cecere et al., 2006; Gürtler et al., 2004; Rabinovich et al., 2011; World Health Organization, 2002). These ecotopes are heterogeneous in size and construction materials; refuge availability for bugs; microclimatic conditions, and resident host species (e.g., humans, dogs, cats, chickens, pigs, and goats).

Weather conditions affect peridomestic bug populations much more than those occupying domestic habitats (Gurevitz et al., 2011; Gürtler et al., 2004; López et al., 1999; Lorenzo et al., 2000; Vázquez-Prokopec et al., 2002). Moreover, the blood meal host species may contribute to

the environmental and nutritional stress that each specific type of habitat exerts on the insects because mammalian and avian blood differ in several respects that affect the haemostatic mechanism (i.e., bird thrombocytes versus mammal platelets) and in other characteristics: hematocrit, viscosity, protein, and water content (Lehane, 2005; Lewis, 1996). In rural villages of Figueroa, in the Argentine Chaco region, bug populations of *T. infestans* occupying distinct habitats differed substantially and consistently in various fitness-related attributes (e.g., blood-feeding rates, host-feeding sources, degree of engorgement and fecundity); chicken coops and domiciles led the ranking of high-quality habitats whereas goat corrals, pig corrals and kitchens were at the other extreme (Gürtler et al., 2014a).

FA has been widely used to infer developmental instability (Palmer and Strobeck, 1986), and its origins are mainly related to environmental factors (Møller and Swaddle, 1997; Parsons, 1990). When asymmetry is slight, random and distributed around a mean of 0, it is often referred to as fluctuating asymmetry (FA). Wing asymmetry may mechanically compromise flight performance and wing function in insect species (e.g., Crespi and Vanderkist, 1997; McLachlan, 1997, 2010). The identified causes of developmental instability in insects include temperature variations, lack of food, and chemical pollution (Bjorksten et al., 2001; Mpho et al., 2001; Talloen et al., 2004; Vishalakshi and Singh, 2008).

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Asymmetry is expected to influence biological performance directly and compromise mechanical functions (Balmford et al., 1993; Dufour and Weatherhead, 1996; Palmer, 1994; but see Leung and Forbes, 1996). Because both sides of bilateral traits develop under the control of an identical genome in the same environment, increased levels of FA may indicate the expression of perturbations accumulated during development (reviewed in Polak, 2003). Resource-limited habitats may constrain the development of an adaptive trait to suboptimal levels, and condition-dependent costs may contribute significantly to observed phenotypic variations in the field.

In Triatominae, higher levels of FA occurred in a domestic rather than in a sylvatic population of *Triatoma sordida* (Dujardin et al., 1999), whereas in *Rhodnius prolixus* under experimental conditions, no differences in the level of FA occurred between simulated sylvan versus domestic conditions (Márquez and Saldamando-Benjumea, 2013). For species within the *Triatoma dimidiata* complex, hybrids had similar FA in wing size and shape as parental groups, and the level of asymmetry in shape varied between villages (Nouvellet et al., 2011). Wing FA usually were greater in males than in females across populations of *T. dimidiata* (Lehmann et al., 2005). To our knowledge, the occurrence of FA in relation to the natural habitats and host-feeding sources of Triatominae has not been investigated.

In this study we measured the occurrence and amount of wing size and shape FA as well as the patterns of asymmetry in *T. infestans* populations collected in five well-defined ecotopes (i.e., domiciles, storerooms, chicken coops, pig corrals, and goat corrals) from three rural communities in Figueroa, for which we also had information on their individual blood meal sources (human, chicken, pig and goat blood) (Gürtler et al., 2014b). Genetic and phenotypic evidence support that *T. infestans* populations are strongly structured, with a high population differentiation detectable at a small spatial scale (Brenière et al., 1998; Schachter-Broide et al., 2004). Based on this evidence, we assumed that the adult triatomines included in this study most likely developed in the habitats where they were collected; did not have differences in factors intrinsic of the development of each sex, and most likely blood-fed on the local host identified. The expected pattern of symmetry is the result of a particular environment (habitat and food source) during nymphal development. We tested the following hypotheses based on background evidence: 1) repeated blood-feeding on hosts differing in blood quality (i.e., mammals and birds) would modify the occurrence and pattern of FA; 2) bugs from more instable, open peridomestic ecotopes (i.e., kitchens, goat corrals and pig corrals) suffering more extreme fluctuations would show higher amounts of FA than bugs from domiciles; 3) patterns of asymmetry across ecotopes for each sex would differ according to habitat stability and adult bug mobility (Ceballos et al., 2005; Gürtler et al., 2014a).

2. Materials and methods

2.1. Study area

Field work was carried out in October–November 2003 in three neighboring rural communities: Barrio Nueva Esperanza (BNE), Bajo Cequeira (BC) and Vaca Huañuna (VH) from Figueroa Department (27° 23' S, 63° 29' W), Santiago del Estero Province, Argentina, described elsewhere (Cecere et al., 2006). The study area was endemic for Chagas disease, with high levels of house infestation with *T. infestans* and reports of acute human cases of *T. cruzi*, and had been sprayed with pyrethroid insecticides by vector control personnel three years before the current study. A cross-sectional survey of house infestation was conducted before conducting a new community-wide insecticide spraying campaign (Cecere et al., 2006). The three selected study communities included 184 houses which had multiple peridomestic structures with specific construction patterns (Cecere et al., 2006). Domiciles, storerooms and kitchens were made of adobe walls and thatched roofs. Chicken coops were built with walls made of wood

sticks or mud bricks and thatched or wood-stick roofs. Goat corrals were usually made of piled thorny shrubs and vertical posts, and pig corrals had solid walls and roofs of wooden planks or trunks.

2.2. Insects

A total of 64 domestic or peridomestic sites (i.e., 20 in BNE; 18 in BC, and 26 in VH) harbored adult *T. infestans* collected by timed-manual searches with a dislodging aerosol (data shown in Gürtler et al., 2014a). All bugs were kept frozen at -20°C upon arrival to the laboratory in Buenos Aires. Identification of the host-feeding sources of each bug was conducted via a direct ELISA against human, dog, cat, chicken, pig, goat and murid rodent (rat or mouse) serum antigens with high sensitivity and specificity values as described (Gürtler et al., 2014a). From the pool of samples we selected all female and male adult insects ($n = 196$) that had unmixed blood meals on a given host species or bug collection habitat for wing asymmetry analysis using geometric morphometry. Bugs that fed on dog, cat, or murid rodent were excluded from the analyses due to their small sample size.

2.3. Metric data

Wings were mounted between microscope slides and cover slips as described in Schachter-Broide et al. (2004). Photographs of each pair of wings were taken using a digital camera (Sony MVC-CD300, US) and a stereo-microscope (Zeiss SV11, Germany). We used landmark-based geometric morphometry. Ten type-I landmarks positioned at vein intersections were collected and expressed as x,y coordinates in Cartesian space. The position of each landmark was described elsewhere (Schachter-Broide et al., 2004).

2.4. Size and shape variation

Centroid size (CS) was used as the size variable for comparison of wing asymmetry between groups. 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).

To obtain Procrustes coordinates, a full Procrustes Analysis superimposition algorithm was applied (Rohlf, 1990; Rohlf and Slice, 1990). The full Procrustes fit analysis optimally translates, rotates and uniformly scales the objects to obtain a similar placement and size by minimizing a measure of shape difference (sum of the squared deviations) between landmarks (Rohlf, 1990).

2.5. Asymmetry assessment

For the analysis of asymmetry (“matching symmetry” according to Mardia et al. (2000), as opposed to “object symmetry”), we first reflected all configurations from one wing side to their mirror images (Klingenberg and McIntyre, 1998; Klingenberg et al., 2002). Separate landmark configurations were digitized twice in images of both wings of each individual for the estimation of measurement error (Palmer, 1994). For each combination of host-feeding source, ecotope and sex, configurations were superimposed by using a full Procrustes fit (Klingenberg and McIntyre, 1998). In addition to possible fluctuating asymmetry (FA), two other types of bilateral asymmetry are expected to occur: directional asymmetry (DA), which occurs whenever there is on average a greater development of a character on one side of the plane of symmetry relative to the other; and antisymmetry (AS), which is detected by a bimodal distribution of signed differences with a zero mean. Unlike DA, FA and AS are non-directional asymmetries. Antisymmetry and DA are believed to have a significant genetic basis (Carter et al., 2009; Palmer and Strobeck, 1986; Pelabón and Hansen, 2008; Van Valen, 1962), and are presumably unrelated to developmental stability (Palmer, 1994).

2.6. Asymmetry of size

To partition asymmetry into directional and non-directional asymmetries, we used a two-way mixed ANOVA with side (fixed) and individual (random) as factors (Palmer and Strobeck, 1986). To examine non-directional asymmetry as an estimate of FA, we tested whether the distribution of the signed differences fitted to a normal distribution (Shapiro–Wilk, kurtosis and skewness statistics). For each insect in each group, the individual amount of FA was estimated using the centered signed right–left differences in wing centroid size (e.g., Kimmerle and Jantz, 2005; Richtsmeier et al., 2005).

2.7. Asymmetry in shape

In order to examine asymmetry of shape at the population level, a Procrustes ANOVA was performed in each relevant group. The Procrustes ANOVA is an adaptation of the two-way mixed ANOVA (Palmer and Strobeck, 1986) applied to each shape variable (Klingenberg and McIntyre, 1998).

Estimates of individual scores for fluctuating shape asymmetry were calculated using the methodology proposed by Klingenberg and Monteiro (2005), in which each individual score is a distance measuring the deviation of its own asymmetry from the mean asymmetry under the hypothesis of an isotropic model (Dryden and Mardia, 1998; Klingenberg and Monteiro, 2005).

In order to analyze and display the pattern of variation of asymmetry in the positions of landmarks, we used a PCA on asymmetric components of shape variation. The first five PCs accounted for most of the variation (91.7%) in the shape asymmetric components. To examine whether the pattern of asymmetry was similar across ecotopes and sexes, we performed a MANOVA test considering the first five principal components (PCs) as dependent variable and ecotopes as independent variables within each sex. A posteriori Hotelling tests were used to compare asymmetric components across ecotopes within each sex.

We analyzed allometry to determine whether size asymmetry had an effect on shape asymmetry using the procedures proposed by Ludoški et al. (2012). First we computed the correlation between individual FA scores for size and the individual FA scores for shape. Second, we analyzed allometry using a multivariate regression. Shape asymmetry (asymmetric component) was regressed on the signed asymmetry of centroid size. The statistical significance of this regression was estimated using a permutation test with 10,000 iterations (Good, 2000)

against the null hypothesis of independence between size and shape. These analyses were done for each host-feeding source and ecotope.

2.8. Software

Landmark digitalizations were done with TPSdig 2.17 (Rohlf, 2013). Morphometric and statistical analyses were done using MorphoJ 1.05f (Klingenberg, 2011), CLIC (<http://mome-clic.com>) and InfoStat (Di Rienzo et al., 2010).

3. Results

3.1. Wing size asymmetry

Descriptive statistics of wing size asymmetry are presented in Table 1. The signed size differences between right and left wings were negative in all cases for host-feeding sources and ecotopes except for females fed on pig or collected from pig corrals. These differences varied from –0.15 to 0.02 for host-feeding source, and from –0.14 to 0.03 for ecotope. The distribution of signed differences between right and left side wings did not depart significantly from normality (Shapiro–Wilk test), reflecting the absence of antisymmetry both for host-feeding sources and ecotopes (Table 1). Therefore, the centered bilateral difference of size was considered to provide relevant information on the FA of size.

Table 2 shows the results from the two-way mixed ANOVA on wing size for host-feeding sources and ecotopes. Goat-fed female bugs and human-fed males showed FA for wing size. When considering ecotopes as a source of variation, results from the two-way mixed ANOVA showed evidence of FA in wing size for females from chicken coops and storerooms and males from pig corrals (Table 2).

3.2. Wing shape asymmetry

Procrustes ANOVAs on wing shape for host-feeding sources are presented in Table 2. For chicken-fed, goat-fed and pig-fed females and males, the FA of wing shape was statistically significant. For human-fed insects, only males showed a significant FA of shape; females did not exhibit any significant FA. The amount of shape FA were significantly different among host-feeding sources for females ($F_{(3,102)} = 5.91, p = 0.001$) but not for males ($F_{(3,89)} = 1.23, p = 0.305$).

Table 1

Descriptive statistics of wing size for the analysis of fluctuating asymmetry according to host-feeding sources, ecotopes and sex in *T. infestans*.

Host-feeding source	Type of ecotope	Sex	No. of individuals	Mean (SD) right wing CS	Mean (SD) left wing CS	Mean R–L signed differences	Normality–Shapiro Wilk's test	Skewness	Kurtosis
Chicken		Female	38	12.10 (0.61)	12.09 (0.64)	–0.01	W = 0.96; p = 0.59	–0.48	0.94
Chicken		Male	38	11.39 (0.42)	11.46 (0.40)	–0.07	W = 0.94; p = 0.21	–0.32	0.32
Goat		Female	19	12.01 (0.60)	12.15 (0.66)	–0.14	W = 0.96; p = 0.74	–0.22	1.75
Goat		Male	12	11.50 (0.59)	11.65 (0.56)	–0.15	W = 0.93; p = 0.49	–0.34	–0.70
Human		Female	24	11.97 (0.61)	12.09 (0.62)	–0.12	W = 0.90; p = 0.07	0.32	0.16
Human		Male	28	11.51 (0.52)	11.60 (0.56)	–0.10	W = 0.95; p = 0.45	0.09	–0.05
Pig		Female	25	12.00 (0.58)	11.98 (0.56)	0.02	W = 0.91; p = 0.10	–0.38	–0.42
Pig		Male	15	11.51 (0.61)	11.56 (0.55)	–0.05	W = 0.97; p = 0.91	0.20	–0.05
	Chicken coop	Female	9	11.91 (0.62)	12.00 (0.69)	–0.09	W = 0.95; p = 0.79	–0.04	–0.97
	Chicken coop	Male	11	11.34 (0.50)	11.38 (0.45)	–0.04	W = 0.98; p = 0.99	–0.39	0.80
	Domicile	Female	31	11.94 (0.63)	12.08 (0.67)	–0.14	W = 0.85; p = 0.07	–1.57	1.32
	Domicile	Male	43	11.45 (0.47)	11.56 (0.52)	–0.11	W = 0.97; p = 0.79	0.16	0.59
	Goat corral	Female	23	12.16 (0.57)	12.22 (0.65)	–0.06	W = 0.89; p = 0.09	0.26	0.64
	Goat corral	Male	13	11.57 (0.42)	11.69 (0.54)	–0.12	W = 0.91; p = 0.34	–0.49	–1.15
	Kitchen	Female	9	11.88 (0.84)	11.92 (0.67)	–0.05	W = 0.83; p = 0.08	0.27	–0.32
	Kitchen	Male	14	11.37 (0.39)	11.37 (0.46)	–0.03	W = 0.98; p = 0.96	–0.35	1.37
	Pig corral	Female	21	12.07 (0.62)	12.04 (0.62)	0.03	W = 0.90; p = 0.09	–0.88	–0.57
	Pig corral	Male	14	11.52 (0.58)	11.53 (0.57)	–0.01	W = 0.96; p = 0.86	–0.51	–0.73
	Storeroom	Female	20	12.20 (0.57)	12.32 (0.63)	–0.12	W = 0.96; p = 0.79	0.27	0.46
	Storeroom	Male	9	11.62 (0.47)	11.70 (0.58)	–0.08	W = 0.93; p = 0.74	0.53	–0.87

Table 2
Two-way mixed ANOVAs and Procrustes ANOVAs to partition asymmetry between directional (MS side) and non-directional (MS side * individual) according to host-feeding source and ecotope for females and males of *T. infestans*.

Host-feeding sources	Ecotope	Sex	n	Size			Shape		
				Side	Side * individual	Measurement error	Side	Side * individual	Measurement error
Chicken		Female	38	0.012	0.090	0.070	0.130**	0.047***	0.011
Chicken		Male	38	0.187*	0.034	0.027	0.156	0.047***	0.000
Goat		Female	19	0.190	0.132*	0.063	0.051	0.074***	0.014
Goat		Male	12	0.126	0.067	0.006	0.032	0.043***	0.006
Human		Female	24	0.076	0.048	0.038	0.026	0.016	0.015
Human		Male	28	0.130	0.089***	0.012	0.078	0.066***	0.010
Pig		Female	25	0.018	0.057	0.044	0.148*	0.074***	0.015
Pig		Male	15	0.007	0.047	0.037	0.227**	0.083***	0.005
	Chicken coop	Female	9	0.016	0.061***	0.006	0.060	0.042***	0.005
	Chicken coop	Male	11	0.026	0.058	0.039	0.080**	0.036***	0.008
	Domicile	Female	31	0.323	0.039	0.063	0.043	0.023	0.002
	Domicile	Male	43	0.711**	0.081	0.055	0.121**	0.051***	0.015
	Goat corral	Female	23	0.000	0.046	0.006	0.071*	0.039***	0.016
	Goat corral	Male	13	0.002	0.064	0.035	0.107*	0.061***	0.007
	Kitchen	Female	9	0.165	0.044	0.029	0.058	0.070***	0.011
	Kitchen	Male	14	0.000	0.057	0.009	0.044	0.036***	0.007
	Pig corral	Female	21	0.015	0.064	0.049	0.144***	0.052***	0.015
	Pig corral	Male	14	0.006	0.064*	0.029	0.136*	0.074***	0.010
	Storeroom	Female	20	0.324	0.098*	0.048	0.068*	0.039***	0.010
	Storeroom	Male	9	0.018	0.031	0.026	0.035	0.027***	0.012

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

Indices of shape FA for host-feeding source showed that females fed on goat and on pig exhibited the largest FA indices (Fig. 1). For males, those fed on pig and human blood showed the largest indices. Human-

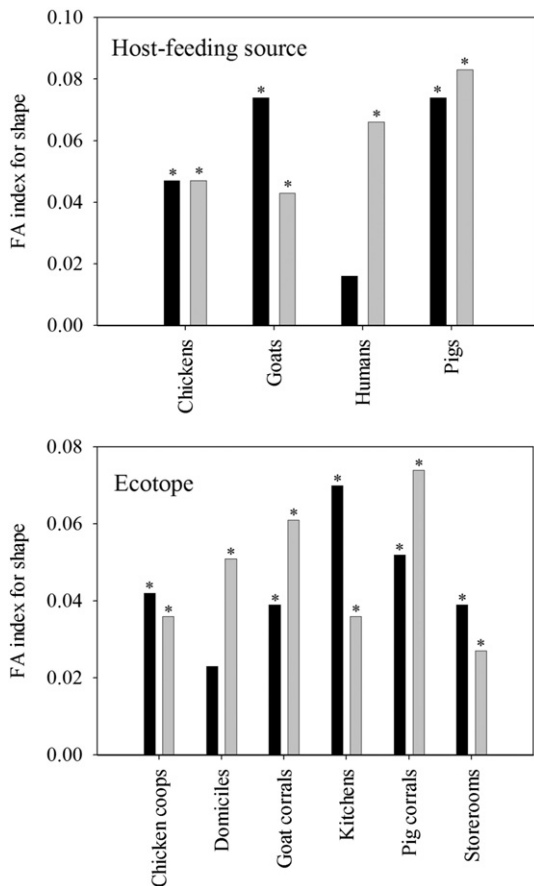


Fig. 1. Fluctuating asymmetry index for wing shape of *T. infestans* for each host-feeding source and ecotope. Females: black bars, males gray bars. The index is the mean square (MS) for the interaction side * individual effect as shown in the ANOVA output of Table 2. Asterisks above bars indicate values statistically significant.

fed females had the lowest indices and the only non-significant ones (Table 2). When considering ecotopes as a source of variation, results from Procrustes ANOVA on wing shape showed evidence of FA in females and males of chicken coops, kitchens, goat corrals, pig corrals, and storerooms (Table 2). For domiciles, only males (not females) showed significant amounts of FA for wing shape (Table 2).

FA indices for wing shape (Fig. 1) showed that females collected from domiciles exhibited the lowest and only non-significant FA index, likewise the results for host-feeding sources (Table 2). Females from kitchens and males from pig corrals exhibited the greatest FA index for shape.

Average differences between right and left wings of bugs fed on different host-feeding sources are shown in Fig. 2. Wing shape varied across blood meal sources, and average differences between wings became evident. Fig. 2 also reveals no obvious differences between right and left wings when the insects were fed on human blood. Females showed more evident shape differences than males across host-feeding sources. Fig. 2 shows that wings from females fed on goat apparently exhibited the greatest asymmetries in shape compared with females fed on other host-feeding sources, although the FA index for shape was the same for goat- and pig-fed females.

Fig. 3 shows that differences in average shape between left and right wings across ecotopes were not as large as for host-feeding sources. Likewise host-feeding sources, wing shape variation across females from different ecotopes was greater than for males.

MANOVA test revealed significant differences in the pattern of asymmetry across ecotopes and sexes (Table 3). The Hotelling a posteriori test revealed three significantly different groups ($p < 0.05$). The first group only included females of goat corrals, whereas the second group included males from all ecotopes and females from chicken coops, kitchens, storerooms and pig corrals. The third group only included females from domiciles (Table 3).

The results of the correlation between size and shape variation of asymmetry are presented in Table 4. Correlations between individual FA scores for shape and size were not statistically significant for any sample studied both for host-feeding sources and ecotopes. Furthermore, multivariate regressions of shape asymmetry on size asymmetry estimates were performed across all samples. Permutation tests indicated that there was no allometric effect of asymmetry except for females of storerooms (Table 4).

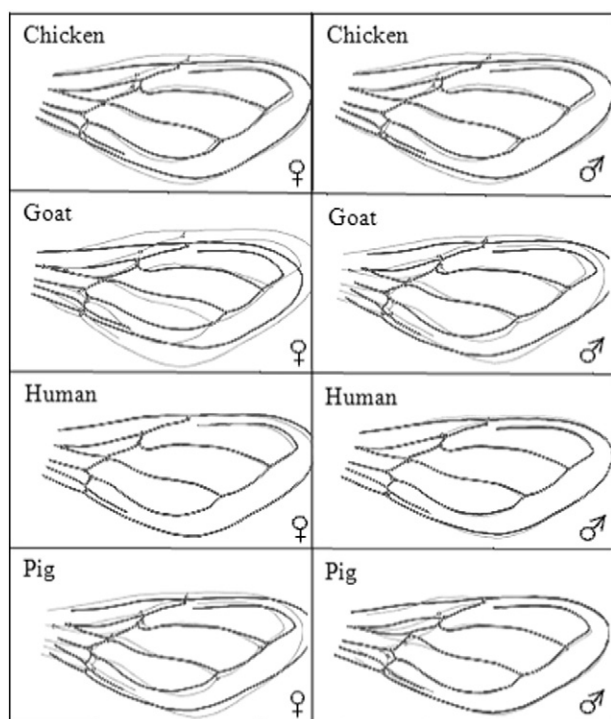


Fig. 2. Average differences between the shape of right and left wings for different host-feeding sources of *T. infestans*. Right wings are in black and left wings in gray. Shape changes correspond to an arbitrary scale factor of 4.0.

4. Discussion

Our study shows evidence of FA both in wing size and/or shape in bugs that blood-fed on chickens, goats and pigs. Goat- and pig-fed female bugs exhibited the largest wing shape asymmetries with respect to other blood meal sources, whereas wing shape FA was absent only in human-fed females. These results suggest that the characteristics of the hosts, host blood composition or the associated host-feeding process may affect wing developmental instability of *T. infestans* as predicted by hypothesis 1. In reference to bug collection ecotope effects, we found evidence of FA in wing shape for males across all habitats, being highest in pig corrals. This result partially supports hypothesis 2; however, males from domiciles showed FA, which was unexpected on the basis of the higher stability and quality of domestic habitats. Variations in shape were consistent for all ecotopes and host-feeding sources except for females fed on humans and from domiciles. Variations in size were only evident for a few host-feeding sources (i.e., goat-fed females, and human-fed males) or ecotopes (i.e., females from chicken coops and storerooms, and males from pig corrals). Host-feeding source and ecotope were the two factors we addressed but their effects on wing developmental instability cannot be teased apart because there was a very strong association between habitats and host species (i.e., bugs collected in a given ecotope fed mostly on the main local host). Therefore, our study portrays the combined effects of habitat and host-feeding source (i.e., environment).

Adults of *T. infestans* collected in distinct ecotopes differed in the amount and pattern of FA. The most likely causes of these variations were ecotope-specific conditions and sex-specific flight dispersal behavior. Females from peridomestic ecotopes exhibited the largest wing shape asymmetries. Peridomestic structures such as goat corrals, pig corrals and chicken coops usually displayed more extreme, widely variable conditions (temperature, relative humidity, and insolation) that may function as environmental stressors for the developing bugs (Vázquez-Prokopec et al., 2002). This is also evident in the differences between left and right wing FA in mean size and shape in bugs from

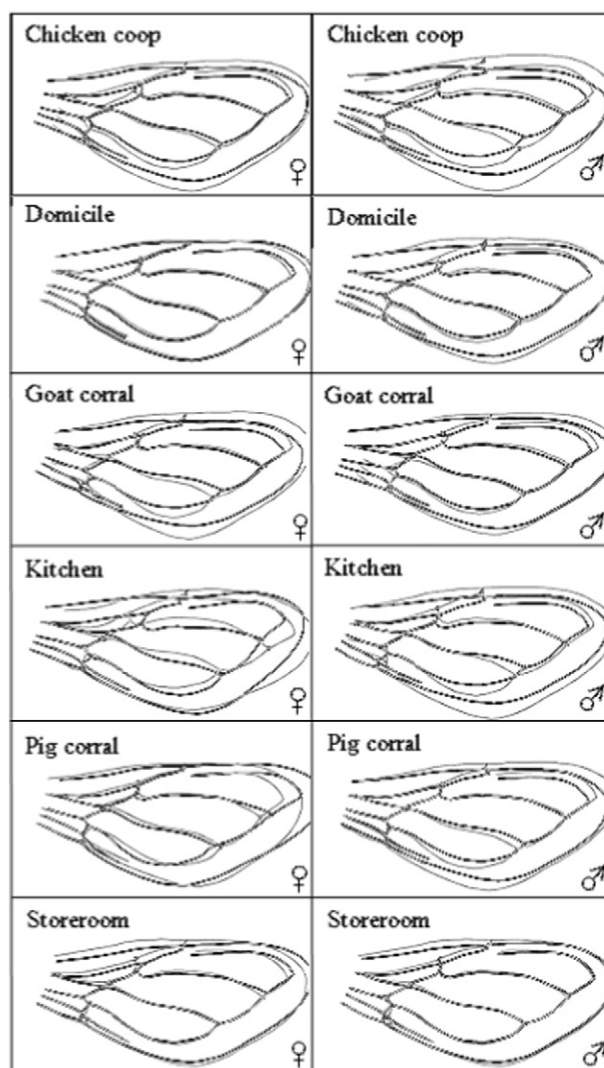


Fig. 3. Average differences between the shape of right and left wings for different ecotopes of *T. infestans*. Right wings are in black and left wings in gray. Shape changes correspond to an arbitrary scale factor of 4.0.

kitchens, goat and pig corrals. Moreover, goats and sheep repeatedly exposed to triatomine bugs or tsetse flies in the insectary developed acquired anti-feeding resistance (Gardiner and Maddrell, 1972; Nash et al., 1965). This host–vector interaction may affect the feeding process and engorgement level of triatomine bugs on previously exposed goats. The large wing asymmetry (mostly differences in size and shape between left and right wings) of goat-fed bugs was evident in females but not in males, which may be attributed to their greater mobility (see below).

The patterns of wing asymmetry across ecotopes, evaluated through MANOVA, showed that females from domiciles and goat corrals differed substantially when compared with females from other habitats, whereas males did not exhibit a distinct pattern across ecotopes. These results, combined with those reported in relation to sex-biased flight dispersal across ecotopes (Ceballos et al., 2005; Vázquez-Prokopec et al., 2006; Gürtler et al., 2014a), identify two lines of relationships. First, females likely have lower mobility than males, and therefore may better reflect local microhabitat conditions and serve as indicators of local environmental stress (e.g., female bugs from domiciles lacked FA). Second, because males are more vagile than females across ecotopes and more so in goat corrals, males most likely have a lower chance of developing and emerging in the site where they were collected and blood-fed on the same local host species. This would explain why males showed

Table 3
MANOVA test performed with the first five PC of the asymmetric components of shape variation according to ecotope for females and males of *T. infestans*.

Type of ecotope	Sex	Effect	MANOVA test			
			Value	F	df	Hottelling a posteriori test (p < 0.05) ^a
		Pillai's trace	0.29	1.21*	55	
		Wilks' lambda	0.71	2.01**	55	
		Hottelling's trace	0.33	1.23*	55	
		Roy's largest root	0.19	3.72***	11	
Chicken coop	Female					b
Chicken coop	Male					b
Domicile	Female					c
Domicile	Male					b
Goat corral	Female					a
Goat corral	Male					b
Kitchen	Female					b
Kitchen	Male					b
Pig corral	Female					b
Pig corral	Male					b
Storeroom	Female					b
Storeroom	Male					b

^a Different letters indicate significant differences between groups.

* p < 0.05.

** p < 0.01.

*** p < 0.001.

wing shape FA across ecotopes, evidenced graphically and statistically, and no significant differences in the pattern of shape FA.

Host-feeding source imply both the nutritional quality of each specific type of blood and the feeding process, which is influenced by host specific factors (e.g., probing time, frequency of contractions of the cibarial pump, and interruptions during the engorgement phase) (Guarneri et al., 2000; Martínez-Ibarra et al., 2003) and the specific

Table 4
Correlations between wing size and shape individual FA scores and multivariate regressions of the asymmetry component (computed from Procrustes coordinates) within each sample of *T. infestans*. Data sets used for each analysis are specified in Table 1. % predicted indicates the amount of size-related shape variation.

Host-feeding source	Type of ecotope	Sex	Correlation coefficient between size and shape individual FA scores		CS FA vs. asymmetric components
			r coefficient	df	Multivariate regression (% predicted)
Chicken		Female	0.14	36	8.20
Chicken		Male	0.06	36	2.12
Goat		Female	0.16	17	4.23
Goat		Male	-0.13	10	6.57
Human		Female	0.08	22	6.02
Human		Male	-0.12	26	1.49
Pig		Female	0.27	23	11.69
Pig		Male	0.03	13	4.09
	Chicken coop	Female	0.03	7	12.52
	Chicken coop	Male	0.19	9	10.51
	Domicile	Female	0.25	29	12.28
	Domicile	Male	-0.32	41	4.82
	Goat corral	Female	-0.10	21	6.57
	Goat corral	Male	-0.05	11	12.38
	Kitchen	Female	0.30	7	21.62
	Kitchen	Male	-0.19	12	6.93
	Pig corral	Female	0.09	19	7.06
	Pig corral	Male	0.12	12	11.60
	Storeroom	Female	0.25	18	18.30**
	Storeroom	Male	-0.03	7	5.00

** p < 0.01.

conditions of each ecotope (i.e., host density, size and construction materials, and microclimatic conditions).

Bugs feeding on lower-quality blood meal sources were expected to display higher levels of FA than those feeding on higher-quality sources (hypothesis 1). In our study, bugs collected from ecotopes used by chickens were mainly fed on chickens and had substantially greater blood-feeding rates and engorgement levels than bugs from domiciles, pig and goat corrals (Gürtler et al., 2014a). The fact that bugs fed on chickens (or collected in chicken coops) exhibited high levels of FA in wing shape suggests that: 1) regardless of the blood-feeding frequency and engorgement status, the quality of blood ingested may affect FA since avian blood has lower nutritional quality than mammalian blood (Lehane, 2005), and 2) The host-feeding process and/or habitat conditions may act as stressor factors for the development of *T. infestans*.

Humans provide high-quality blood meals for triatomine bugs (Aldana et al., 2009; Gomes et al., 1990). Unlike other hosts, the composition of human blood includes lipids, which are used for flight metabolism (Canavoso et al., 2003); a high concentration of proteins, which are the most nutritious blood elements, and a low percentage of water content (Lehane, 2005). In agreement with these attributes, experimentally-fed *R. prolixus* bugs fed on human or rabbit blood throughout their entire life course showed reduced development times and increased fecundity relative to those fed on chicken, sheep or horse blood (Gomes et al., 1990). Furthermore, domestic ecotopes had near-optimum temperatures for *T. infestans* and more capacity to damp variations in external temperatures and relative humidity than other frequently infested peridomestic ecotopes (Vázquez-Prokopec et al., 2002). Humans as a blood meal source and domiciles as habitats were the only ones that were not associated with FA in female wing size or shape.

The interpretation of our results is limited by aspects related to the study design. First, the sample size for some of the study groups was below the suggested minimum of 30 individuals (Polak, 2003), which may affect some comparisons between and within ecotopes in which adult bug abundance was limited (e.g., kitchens, goat and pig corrals). Second, although we cannot ensure that adult bugs from a given site fed on the same local host species throughout their development, most of the study sites harbored established bug colonies that developed during the fall-early spring period, when temperature-dependent flight dispersal is minimal (Gürtler et al., 2014a). Furthermore, the pattern of shape FA across ecotopes was generally consistent with that observed for host-feeding sources. Third, the three study communities had been sprayed with pyrethroid insecticides three years before the bug collection survey. It is highly unlikely that the insecticide campaign may have exerted any direct effects on wing symmetry because: 1) FA is often used as a measure of the developmental stability of an organism and with null heritability (Palmer, 1994), and 2) the egg-to-adult development period of *T. infestans* may average six months (Rabinovich, 1972), while the residual effects of pyrethroids on various peridomestic substrates only last for 1–2 weeks (Gürtler et al., 2004). Previous insecticide spraying campaigns had also been sporadic.

5. Conclusions

Our study provides three lines of evidence for wing developmental instability related to the combined influence of ecotopes and host-feeding sources: 1) wing size asymmetry appears to be insensitive to the amount of FA across ecotopes, host-feeding sources and sexes. Size FA was only evident for a few host-feeding sources or ecotopes; 2) variations in wing shape FA are more related to host-feeding sources, sexes and ecotopes than wing size FA. Variations in shape FA were consistent across host-feeding sources and ecotopes except for females fed on humans and from domiciles; and 3) the amount and pattern of FA differed between ecotopes and sexes, in agreement with the observed differences in sex-specific dispersal behavior. Females had a remarkably different pattern of asymmetry in domiciles and goat corrals compared

with other habitats. The relationship between development and wing functional performance in *T. infestans* and other Triatominae still remains to be investigated.

Acknowledgments

We thank María C. Cecere, Leonardo A. Ceballos, Juan M. Gurevitz and Gonzalo M. Vázquez-Prokopec for providing biological materials used in this study. Julieta Nattero and Ricardo E. Gürtler are members of the CONICET Researcher's Career. This study was supported by awards from University of Buenos Aires (UBACYT 2011–2014), Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 2012–2015), and Agencia Nacional de Promoción Científica y Tecnológica (PICT 2011–2012, PICTO-Glaxo 2011–0062). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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