



## Research paper

# Temporal variations of fluctuating asymmetry in wing size and shape of *Triatoma infestans* populations from northwest Argentina



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## ARTICLE INFO

## Keywords:

Developmental instability  
Dispersal  
Geometric morphometry  
Habitat heterogeneity

## ABSTRACT

Environmentally-induced developmental instability has frequently been assessed using fluctuating asymmetry (FA) methods. For *Triatoma infestans*, the major vector of Chagas disease, the combined effects of host-feeding sources and habitats affected wing developmental instability depending on sex in Figueroa villages, northwest Argentina. Here we investigated whether habitat, sex, season/year and insecticide applications affected wing developmental instability in *T. infestans* populations from Amamá and other rural villages of northwest Argentina over a four-year period. We measured the occurrence and amount of wing size and shape FA in 423 adult triatomines collected in domiciles, goat corrals, pig corrals, storerooms and wood piles. Significant wing size and wing shape FA occurred in females and males from all habitats as determined by two-way mixed ANOVA and Procrustes ANOVA, respectively. For wing size and shape, the highest corrected indices of FA (FAI) for females occurred in wood piles, goat corrals and domiciles in late summer or early autumn, whereas for males, the largest FAIs consistently appeared in domiciles and storerooms. Wing size FAIs were significantly higher in recently infested goat corrals rather than in persistently infested goat corrals. The follow-up of four infested peridomestic sites showed that FA patterns were not stable over time or sites. Temporal variation of FA among habitats appears to be modified by the history of insecticide spraying, either through direct effects on insect development or through indirect effects related to flight dispersal and house invasion. Whether FA may provide another marker to identify the sources of reinfestant triatomines requires further investigation.

## 1. Introduction

*Triatoma infestans* (Hemiptera, Reduviidae) is the main vector of Chagas disease in southern South America. This species is well-adapted to thrive in human sleeping quarters and other human-made or -modified structures used for domestic animals (Gürtler et al., 2014a, 2014b). The peridomestic habitats occupied by *T. infestans* substantially differed in size, construction materials, microclimatic conditions, refuge availability, host species and host numbers (Ceballos et al., 2005; Cecere et al., 2004; Vazquez-Prokopec et al., 2002). The adaptation and epidemiological importance of *T. infestans* justified targeting this species for elimination in the southern cone countries of South America since 1991 (World Health Organization (WHO), 2017). Insecticide spraying reduced the geographic range and abundance of *T. infestans* but did not interrupt the vector-borne transmission of human *Trypanosoma cruzi* infection, the etiological agent of Chagas disease, in the Gran Chaco region of Argentina, Bolivia and Paraguay (World Health

Organization (WHO), 2017).

Flight dispersal is one of the mechanisms driving house invasion and recolonization after control interventions directed against *T. infestans* (Schofield and Matthews, 1985). The flight dispersal of this species in the dry Argentine Chaco would peak in summer, vary substantially among spring seasons (Di Iorio and Gürtler, 2017), and remain at low levels in late fall and winter, with contrasting patterns between female and male *T. infestans* (Gurevitz et al., 2006; Vazquez-Prokopec et al., 2006). Female-biased flight dispersal of *T. infestans* populations has been revealed using genetic and morphological markers (Gaspé et al., 2012; Marcet et al., 2008; Pérez de Rosas et al., 2013).

Fluctuating asymmetry (FA) has frequently been used as a measure of environmentally-induced developmental instability (reviewed in Beasley et al., 2013). FA levels both in size and shape generally increased in insect populations subjected to environmental stressors such as extreme variations in temperature, lack of food, and chemical pollution (Beasley et al., 2013). In Triatominae, FA differed between

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domestic and sylvatic populations of *Triatoma sordida* (Dujardin et al., 1999) and between sexes across villages in *Triatoma dimidiata* (Lehmann et al., 2005; Nouvellet et al., 2011). Conversely, no differences in FA levels were found in *Rhodnius prolixus* kept under simulated sylvatic and domestic conditions (Márquez and Saldamando-Benjumea, 2013), and between hybrids and parental groups in the *T. dimidiata* complex (Nouvellet et al., 2011).

For *T. infestans* populations, wing developmental instability was significantly associated with its host-feeding sources and habitats depending on sex in rural villages of Figueroa in the Argentine Chaco (Nattero et al., 2015). These villages had been last sprayed with pyrethroid insecticides three years before the vector survey. Therefore, we assumed that adult triatomines collected in early spring most likely had developed in the habitats where they were caught, and the observed patterns of symmetry resulted from the particular combination of habitat and host blood source during development.

Here we investigated the occurrence and amount of temporal variations of wing size and shape FA of *T. infestans* populations in Amamá and other neighboring rural communities, which are approximately 50 km away from Figueroa villages. In Amamá villages, selective insecticide sprays conducted asynchronously determined a particular dynamics of house invasion and establishment of *T. infestans* (Gürtler et al., 2007), leading to admixed populations across domestic and peridomestic habitats over the years 2000–2004. These populations displayed temporal variations in wing size and shape congruent with the hypothesis that adults dispersed from goat corrals to domiciles during the warm season (Schachter-Broide et al., 2004, 2009). We inferred that (i) triatomines from a given site that remained infested over a substantial time period would have more in common (genetically and environmentally) than dispersant adults with more variable genetic composition and prior developmental conditions, and (ii) reinfestants would be more likely to originate from less suitable or more unstable habitats, as these would enhance vector emigration. In the specific context of these studies, we expected that Amamá patterns of wing size and shape FA would not be fully consistent with Figueroa patterns, and tested whether *T. infestans* adults from recently infested habitats would have more FA than those from persistently infested ones.

## 2. Materials and methods

### 2.1. Study site

Fieldwork was carried out in the rural villages of Amamá, Trinidad, Mercedes and San Pablo (27°12'33"S, 63°02'10"W), Moreno department, Santiago del Estero province, Argentina, in March 2000, October 2002 and April 2004 (Fig. 1A–C). The three villages were situated within 8 km of each other on semiarid hardwood, thorny forest habitat (Cecere et al., 2006). Most houses had adobe walls and thatched roofs, and the peridomestic area consisted of 3–8 peridomestic structures separated from human habitations (Ceballos et al., 2005) (Supplementary material 1). Storerooms were used by dogs, cats and nesting chickens, and were made of mud-brick or mud-stick, unplastered walls and thick thatched roofs. Goat corrals usually had a fence made with piled thorny shrubs and vertical posts, and a small thatched enclosure. Pig corrals usually had solid walls and roofs made of wooden planks or thatch. Wood piles were located within the area of human activity; residents eventually used the firewood and hence these habitats did not persist for > 1.5 years (Vazquez-Prokopec et al., 2005).

The history and impact of vector control actions in the study villages was described elsewhere (Gürtler et al., 2007). Regular vector surveillance and control actions were undertaken by householders from 1997 up to March 2000; then gradually decreased from March 2000 to October 2002, and stopped between October 2002 and March 2004. All houses and outbuildings were re-sprayed with pyrethroid insecticides by vector control personnel in April 2004.

We report the number of sites that were infested, colonized and

sprayed with insecticides for each of the occasions in which triatomines were collected. Vector survey at time t-1 was taken as the last survey conducted before time t (i.e., for March 2000, the immediately preceding survey occurred in May 1999; for October 2002, in March 2000, and for April 2004, in March and July 2003).

### 2.2. Insects

A total of 153 *T. infestans* adults collected from domestic and peridomestic structures in March 2000, 112 in October 2002, and 158 in April 2004, were included in this study. On each of these surveys, skilled bug collectors from the Chagas disease national vector control program searched for triatomines in all bedroom and peridomestic areas using 0.2% tetramethrin to dislodge the insects (Icona, Buenos Aires, Argentina). One or two men searched bedrooms (0.5 person-hour) while another man searched peridomestic sites during 30 min per house (0.5 person-hour per house). If more triatomines appeared after finishing the search time, they were collected and registered as “post-bug collections”. Three collectors searched for triatomines in 117 houses including 525 identified peridomestic sites in March 2000; 126 houses including 453 peridomestic sites in October 2002, and 126 houses including 109 selected peridomestic sites in April 2004, when only two bug collectors conducted the searches. Bugs were identified to species and stage at the field laboratory as described elsewhere (Canale et al., 2000), and stored at –20 °C. Triatomine habitats included in the current study were domiciles, goat corrals, pig corrals and storerooms. Woodpiles were infested with adult triatomines in March 2000 only. Chicken coops were rare in these villages and therefore were not included in the study.

In addition to ecotope-specific FA patterns aggregated community-wide, we compared adult triatomines from goat corrals that remained infested from October 2002 to April 2004 with those that appeared newly infested in April 2004. This analysis was not performed for other study ecotopes because there were not enough adult insects from persistently infested sites. We also analyzed triatomines from three goat corrals (a24, a34 and a45) and a pig corral (a44) from Amamá that remained infested during a four-year period March 2000, October 2002, March 2003 and April 2004 (Fig. 1A).

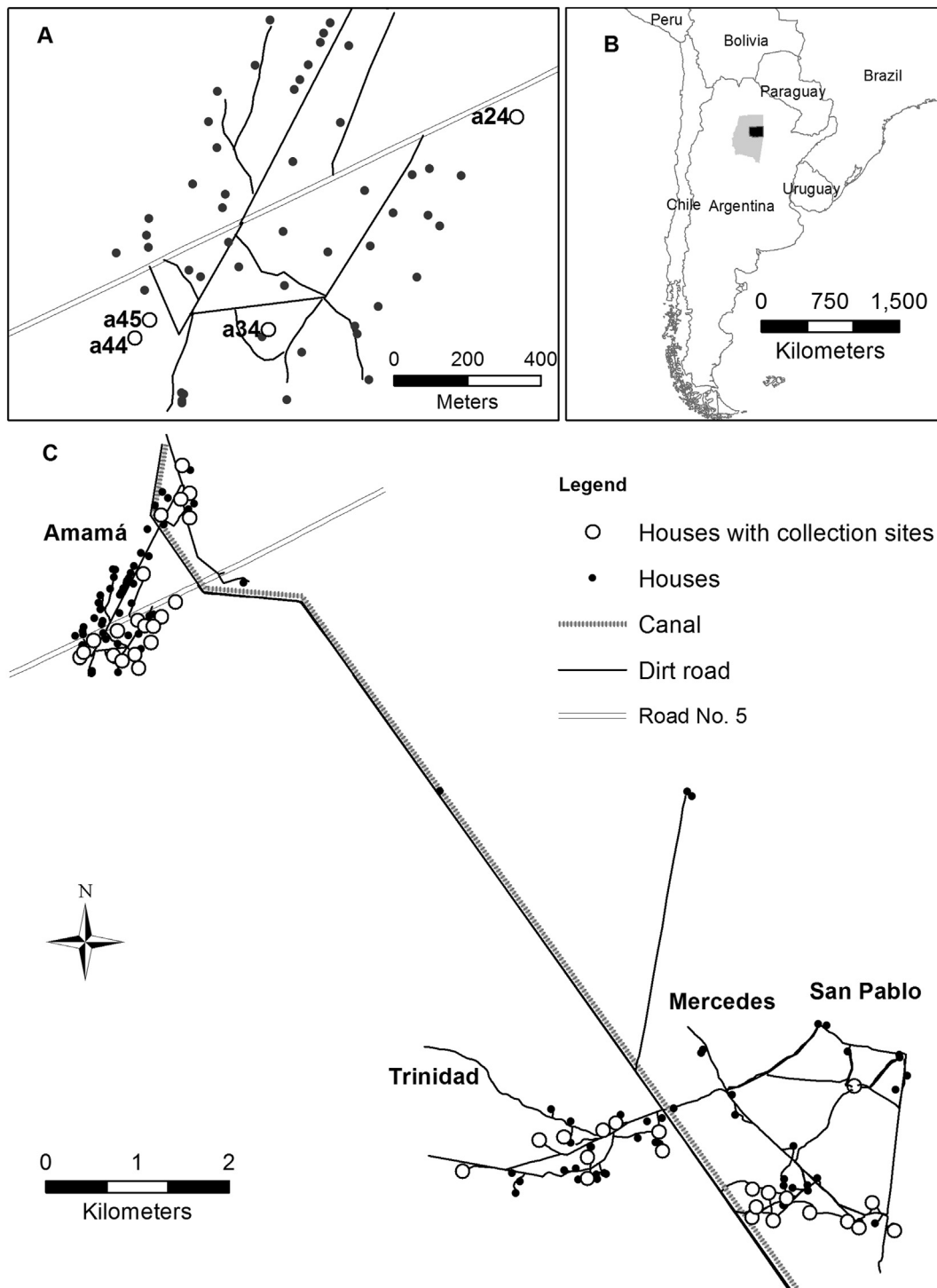
### 2.3. Wing data collection

Both wings were mounted between microscope slides and cover slips as described in Schachter-Broide et al. (2004). Photographs were taken using a digital camera (Sony MVC-CD300, US) and a stereo-microscope (Zeiss SV11, Germany) using a 6 × magnification. We used landmark-based geometric morphometry to collect 10 type-I landmarks positioned at vein intersection as described elsewhere (Schachter-Broide et al., 2004) (Supplementary material 2). Landmark collection was done with TPSdig 2.17 (Rohlf, 2013). Using this technique we obtained wing size and shape variables to compare asymmetry between groups.

For assessing the size asymmetry of a morphological structure, the centroid size (CS) can be computed from landmark configurations of the left and right sides of each individual. 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). For wing shape, the comparison of left and right sides was done with a Procrustes approach, by computing differences of landmark coordinates after a Procrustes superimposition of both sides' configurations.

### 2.4. Asymmetry assessment

All left wings were first reflected to their mirror images. Separate landmark configurations were digitalized twice in both wings of each individual to estimate measurement error (ME) (Palmer, 1994).



**Fig. 1.** Map of the study area including location of houses with collection sites from the rural villages of Amamá, Trinidad, Mercedes and San Pablo. (A) Detail of Amamá village with the location of the four studied sites (a24, a34, 44 and a45); (B) location of Moreno department, Santiago del Estero province (gray), in Argentina; and (C) houses with collection sites included in this study.

Configurations for each type of ecotope, sex and collection year were superimposed by using the least-squares Procrustes method (Klingenberg and McIntyre, 1998).

Asymmetry can be partitioned in directional and non-directional asymmetries. Directional asymmetry (DA) occurs whenever there is, on average, a greater development of a character on one side of the plane of symmetry relative to the other. DA is presumably unrelated to developmental stability (Palmer, 1994). Non-directional asymmetry measures FA. A two-way mixed ANOVA with side (fixed) and individual

(random) as factors was used for each ecotope, sex and triatomine collection year to assess the occurrence of DA and FA (Palmer and Strobeck, 1986).

Shapiro–Wilk tests were performed to evaluate the normality of the distribution of signed differences between right and left wings for each group (ecotope within each sex and triatomine collection year). Kurtosis and skewness were not statistically significant in any of the groups, reflecting the absence of antisymmetry (Supplementary material 3).

The FA index for size is estimated as the mean square (MS) of the interaction between side and individual of the two-way ANOVA. These indices were corrected for measurement error (MS/ME) in all cases. Fisher's exact tests were used for pairwise comparisons of corrected FA indices for size of all relevant groups (i.e., sexes within each year, ecotopes across years, and ecotopes within each year).

A Procrustes ANOVA was performed in each relevant group to estimate asymmetry in shape. This ANOVA is an adaptation of the two-way mixed ANOVA (Palmer and Strobeck, 1986) applied to each shape variable (Klingenberg and McIntyre, 1998). The FA index for shape is estimated as the mean square (MS) of the interaction between side and individual of the Procrustes ANOVA. These indices were corrected for measurement error ( $MS \times 10^3/ME \times 10^3$ ). To compare corrected FA indices for shape, Fisher's tests were used for pairwise comparisons of all relevant groups.

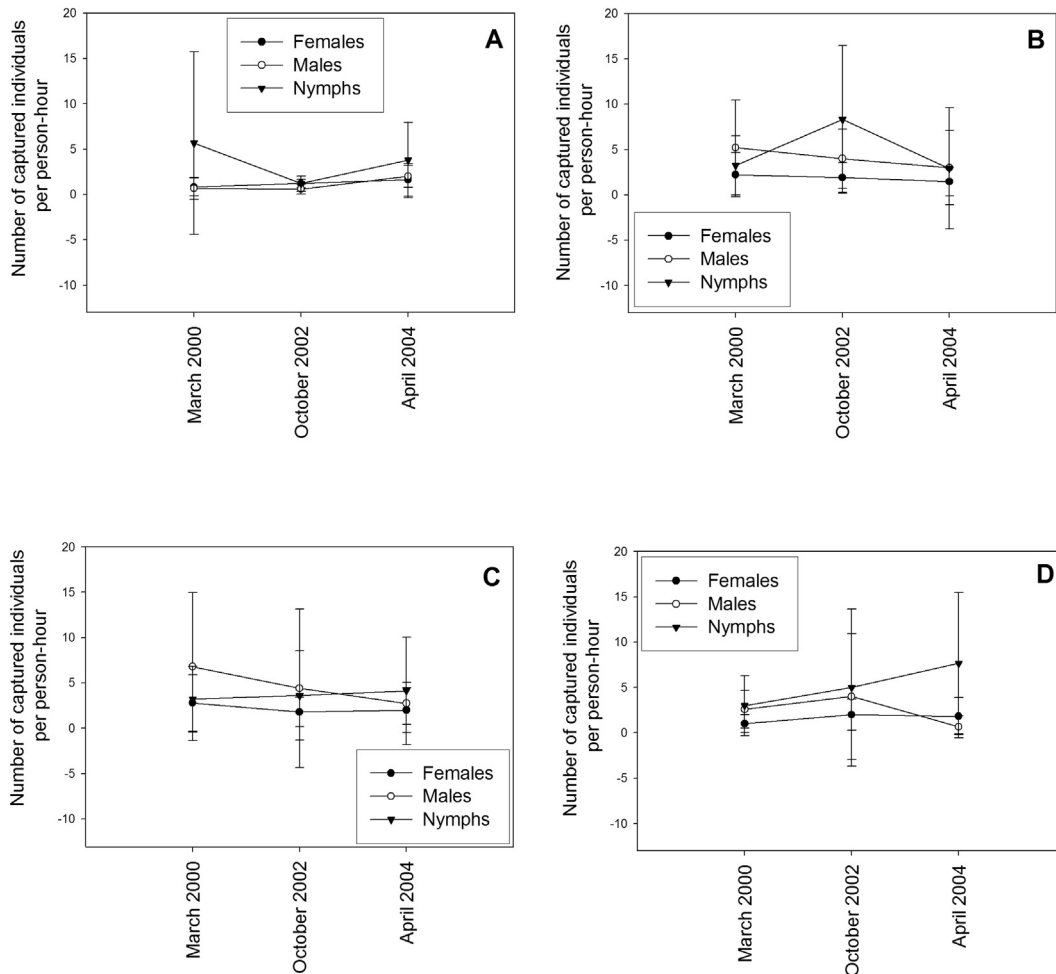
To analyze allometry we computed the correlation between individual FA scores for size and individual FA scores for shape for each ecotope and triatomine collection year within each sex (i.e., sexes within each year, ecotopes across years, and ecotopes within each year). Morphometric and statistical analyses were done using MorphoJ 1.05f (Klingenberg, 2011), CLIC (<http://mome-clic.com>) and InfoStat (Di Rienzo et al., 2016).

**Table 1**

Number of sites infested or colonized with *T. infestans*, and sprayed with pyrethroid insecticides; t-1 is taken as the immediately preceding vector survey.

Survey date	Ecotope	N° of infested study sites	N° (%) of colonized sites	N° (%) of infested sites at t-1	N° (%) of sprayed sites	N° (%) of sprayed sites at t-1
March 200-0	Domicile	5	4 (80)	0 (0)	0 (0)	0 (0)
	Goat corral	9	7 (78)	4 (44)	0 (0)	1 (11)
	Pig corral	4	2 (50)	2 (50)	0 (0)	1 (25)
	Storeroom	5	4 (80)	3 (60)	0 (0)	0 (0)
	Total	23	17 (74)	9 (39)	0 (0)	2 (9)
October 200-2	Domicile	6	5 (83)	1 (17)	0 (0)	0 (0)
	Goat corral	12	11 (92)	6 (50)	1 (8)	3 (25)
	Pig corral	5	4 (80)	3 (60)	2 (40)	3 (60)
	Storeroom	3	1 (33)	1 (33)	1 (33)	1 (33)
	Total	26	21 (81)	11 (42)	4 (15)	7 (27)
April 200-4	Domicile	7	5 (71)	2 (29)	1 (14)	2 (29)
	Goat corral	13	8 (62)	8 (62)	0 (0)	4 (31)
	Pig corral	8	5 (63)	5 (63)	0 (0)	4 (50)
	Storeroom	6	5 (83)	0 (0)	0 (0)	2 (33)
	Total	34	23 (68)	15 (44)	1 (3)	12 (35)

Percentages were estimated with respect to the number of infested study sites.



**Fig. 2.** Mean relative abundance of *Triatoma infestans* (per person-hour) in all study sites according to insect stage, main ecotope and survey year in Amamá and neighboring rural villages. A. Domiciles. B. Goat corrals. C. Pig corrals. D. Storerooms.

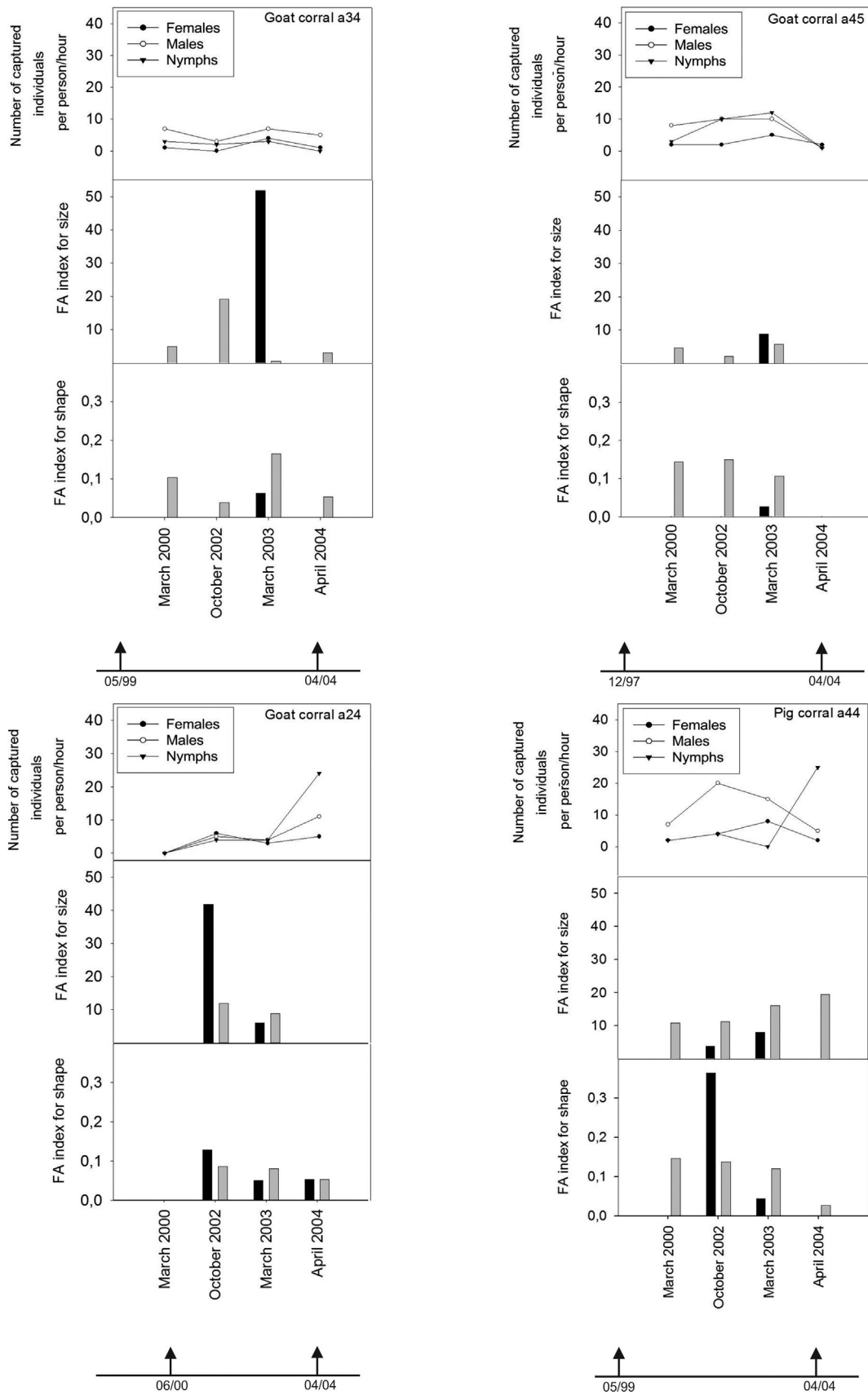


Fig. 3. Mean relative abundance of *Triatoma infestans* (per person-hour) and fluctuating asymmetry index (FAI) for wing size and wing shape of female and male captured in three goat corrals (a34, a45 and a24) and one pig corral (a44) in March 2000, October 2002, March 2003 and April 2004. Dates under each pair of graphs show when insecticide sprays were carried out at each site. Females: black bars; males: gray bars. The index is the mean square (MS) for the side-individual interaction effect corrected for measurement error (MS/ME).

### 3. Results

#### 3.1. House infestation and vector control actions

All the study ecotopes harbored relatively small-sized *T. infestans* populations over time (Fig. 2A–D). Peridomestic ecotopes had much more triatomines than domiciles. For the studied sites, the highest colonization rate occurred in October 2002, when 81% of all inspected sites included at least nymphs (Table 1). Conversely, in March 2000 and April 2004, 26% and 32% of all inspected sites only had adult triatomines, respectively.

Regarding the history of infestation and vector control actions prior to each triatomine survey, nine (39%) of the 23 sites included in March 2000 had previously been infested with *T. infestans* and two had been sprayed with pyrethroids. Of the 26 sites included in October 2002, 11 (42%) had previously been infested and seven (27%) of the former were sprayed with pyrethroids. Of 34 sites included in April 2004, 15 (44%) had previously been infested and 12 (35%) of the former were sprayed with pyrethroids (Table 1).

Following the insecticidal treatments, only males were subsequently collected at the a34, a44 and a45 corrals (Fig. 3). Both females and males were caught at the a24 goat corral over the following surveys. Site colonization (i.e., presence of nymphs) was registered whenever female triatomines were collected concurrently.

#### 3.2. Wing size asymmetry

Significant evidence of FA in wing size was revealed by the two-way mixed ANOVAs for ecotope, sex and year/season of bug collection (Tables 2, 3 and 4). Females showed wider variability in corrected fluctuating asymmetry indices (FAI) than males in the four main ecotopes. Females from goat corrals and wood piles collected in March 2000 showed the highest FAIs over all study sites and occasions (Fig. 4A), and differed significantly from females collected in other ecotopes in March 2000, October 2002 and April 2004 (Fisher's test,  $p < 0.0001$  in all cases). Females from pig corrals and domiciles showed maximal FAI for wing size in October 2002 and April 2004, respectively (Fig. 4A). Males from domiciles and storerooms showed maximal FAIs over the study period whereas males from goat corrals exhibited the least variable FAI (Fig. 4A). No pairwise comparison of FAI for either sex showed significant differences in October 2002 and April 2004.

We compared wing size FA in triatomines from goat corrals that either remained infested from October 2002 to April 2004 or first appeared infested in April 2004. Wing size FAIs were significantly higher in recently infested corrals than in persistently infested corrals both in females (corrected FAIs: 1.362 and 0.044, respectively, Fisher's test,

$p < 0.0001$ ) and males (1.711 and 0.099, respectively, Fisher's test,  $p < 0.05$ ).

Triatomines captured at the three goat corrals and the pig corral showed significant wing size FA for sex and year in all cases (two-way mixed ANOVAs, Supplementary material 4). FAIs at each corral were widely variable among years, with significant pairwise comparisons in most cases (Fig. 3). Males from the a44 pig corral were the only ones that did not display significantly different wing size FA among years (Fisher's test,  $p > 0.05$  in all cases). Females from the a24 goat corral collected on the survey that followed insecticide treatment had significantly larger wing size FAIs than females collected on other occasions (Fisher's tests,  $p < 0.01$  for all cases). Females from the a34 goat corral collected three years after insecticide spraying (March 2003) showed a large amount of FA (Fig. 3). For the a44 pig corral, females were collected for the first time in October 2002 (two years after insecticide spraying), and displayed a significantly lower amount of wing size FA compared with first-collected females in the other three goat corrals.

#### 3.3. Wing shape asymmetry

Results from the Procrustes ANOVA for wing shape showed that FA was statistically significant for each ecotope, sex and collection year (Tables 2, 3 and 4), as was for wing size. Females from wood piles exhibited the largest FAIs in March 2000 (Fig. 4B). Fisher's tests for all pairwise comparisons showed significant differences only for wood piles ( $p < 0.01$  for all cases). The highest FAIs were detected in females from domiciles in October 2002 and April 2004; in males, maximal FAIs were detected in domiciles in years 2000 and 2002 and in storerooms in year 2004. FAIs varied widely among occasions. Triatomines from persistently infested goat corrals versus recently infested goat corrals in April 2004 showed no significant differences in FAIs (females: 1.255 and 1.158, respectively, Fisher's test,  $p > 0.05$ ; for males: 0.927 and 0.912, respectively, Fisher's test,  $p > 0.05$ ).

When the occurrence of FA was assessed in triatomines from the three goat corrals (a34, a45, a24) and a pig corral (a44) over time, the Procrustes ANOVAs for each corral, sex and year were significant in all cases (Supplementary material 4). Likewise for wing size, wing shape FAIs in corrals was highly variable among years (Fig. 3). Pairwise comparisons showed significant differences between years for males and between females and males within the same year. For goat corrals, males captured at the same site in different years exhibited significantly different FAIs. For the a44 pig corral, females from October 2002 and March 2003 showed significant differences in FAI in relation to males ( $p < 0.001$ ) (Fig. 3).

**Table 2**

Results from mean squares ( $\times 10^3$  for shape) of two-way mixed and Procrustes ANOVAs to partition directional (MS side) and non-directional (MS side \* individual) asymmetry according to ecotope for females and males of *T. infestans* collected in Amamá and neighboring villages in March 2000.

Ecotope	Sex	No. of insects	Size			Shape		
			Side	Side × individual	Measurement error	Side	Side × individual	Measurement error
Domicile	Female	12	27.429	78.643***	0.450	0.024	0.038***	0.000
Domicile	Male	10	197.863	210.483***	0.588	0.051	0.063***	0.001
Goat corral	Female	22	144.101	136.193***	0.130	0.144	0.038***	0.000
Goat corral	Male	23	0.708	23.852***	0.330	0.036*	0.040***	0.000
Pig corral	Female	20	81.826	41.116***	0.428	0.077*	0.031***	0.000
Pig corral	Male	23	74.598	94.792***	0.422	0.066	0.046***	0.000
Storeroom	Female	10	0.325	69.405***	0.573	0.048	0.045***	0.000
Storeroom	Male	13	10.054	40.126***	0.591	0.024	0.054***	0.000
Wood pile	Female	09	19.96	254.230***	0.196	0.029	0.043***	0.001
Wood pile	Male	11	2.723	103.02***	1.257	0.072	0.068***	0.000

\*  $p < 0.05$ .

\*\*\*  $p < 0.001$ .

**Table 3**

Results from mean squares ( $\times 10^3$  for shape) of two-way mixed and Procrustes ANOVAs to partition directional (MS side) and non-directional (MS side \* individual) asymmetry according to ecotope for females and males of *T. infestans* collected in Amamá and neighboring villages in October 2002.

Ecotope	Sex	No. of insects	Size			Shape		
			Side	Side $\times$ individual	Measurement error	Side	Side $\times$ individual	Measurement error
Domicile	Female	9	0.968	52.173***	0.434	0.067	0.085***	0.001
Domicile	Male	9	280.342*	49.683***	0.462	0.026	0.037***	0.001
Goat corral	Female	17	27.151	67.360***	1.161	0.099*	0.040***	0.002
Goat corral	Male	23	56.614	25.143***	0.549	0.046*	0.028***	0.001
Pig corral	Female	12	69.219	109.630***	0.429	0.054	0.044***	0.001
Pig corral	Male	18	47.766	28.238***	0.491	0.016	0.028***	0.001
Storeroom	Female	12	20.277	76.781***	0.862	0.068	0.092***	0.003
Storeroom	Male	12	44.908	41.993***	1.188	0.049	0.054***	0.002

\*  $p < 0.05$ .

\*\*\*  $p < 0.001$ .

### 3.4. Allometric analysis

The correlation coefficients between individual FA scores for wing size and wing shape were not statistically significant for most of the groups examined except for pig corral females and males in year 2000 and females from domiciles in 2002 and 2004 (Table 5).

Correlation analysis was used to assess allometry within each individual corral; wing size and shape FA scores did not show a statistically significant association in all cases (Supplementary material 5).

## 4. Discussion

The *T. infestans* populations included in this study originated from a collection of identified habitats and sites with known specific histories of colonization and insecticide treatment. These characteristics most likely determined the occurrence of admixed populations including established and recent in-migrant adult triatomines, in a context of increasing peridomestic (re)infestation over four years combined with first decreasing (2000 – 2002) and then increasing (2002–2004) domestic infestation (Fig. 2 in Gürtler et al., 2007).

Our results show that temporal variation in wing size and wing shape FA is congruent with habitat/host combinations and/or timing of flight dispersal as predicted. Females from wood piles, goat corrals and domiciles in summer and early autumn and males from domiciles and storerooms exhibited the highest amount of FA both for wing size and shape. The specific local conditions varied greatly among ecotopes and may function as environmental stressors for developing bugs. Wood piles were unstable ecotopes since firewood was gradually consumed, and did not persist for > 1.5 years. Wood piles were eventually used as a resting place by chickens, dogs and other domestic animals on which triatomines fed upon. Goat corrals usually displayed extreme, widely variable temperatures and host numbers than other ecotopes (Ceballos

et al., 2005; Vazquez-Prokopec et al., 2002). These unstable local conditions likely affect both wing asymmetry and flight dispersal propensity, which most likely turn wood piles and goat corrals as sources of flight dispersants with high levels of wing FA.

Males from domiciles and storerooms exhibited the highest FAIs for both wing size and shape in late summer or early autumn (March 2000 and April 2004, respectively). Adult *T. infestans* collected in summer or early autumn were expected to have higher probabilities of having developed and emerged elsewhere than adults collected in early spring; the latter most likely had developed where they were collected because bug movement is very unlikely during the cold season and under 16 °C (Ceballos et al., 2005; Di Iorio and Gürtler, 2017; Vazquez-Prokopec et al., 2006), leaving a narrow time window for flight dispersal as determined by suitable weather conditions (Gürtler et al., 2014a). These patterns coincide with the observed colonization rates, where a higher frequency of sites with adult triatomines only was registered in late summer or early autumn than in early spring (October 2002).

The temporal variation in the occurrence and amount of FA in Amamá differed from the patterns recorded in Figueroa villages over the same season. In Figueroa villages, wing size asymmetry was insensitive to the amount of FA across ecotopes whereas wing shape FA exhibited different patterns (Nattero et al., 2015). Wing shape FA for females from pig corrals or goat corrals in spring (October) was similar in Amamá and Figueroa, whereas females from domiciles displayed a much smaller amount of FA in Figueroa than in Amamá. Two factors may account for these differences. First, the reinfestation dynamics between and within house compounds differed between study areas. In Figueroa villages, *T. infestans* populations had become established and were not under insecticide treatment. Taking FA as a measure of developmental instability, the wing size FA may be canalized (i.e., a developmental buffering against the effects of variation in environmental conditions, Klingenberg, 2015). In Amamá villages, however,

**Table 4**

Results from mean squares ( $\times 10^3$  for shape) of two-way mixed and Procrustes ANOVAs to partition directional (MS side) and non-directional (MS side \* individual) asymmetry according to ecotope for females and males of *T. infestans* collected in Amamá and neighboring villages in April 2004.

Ecotope	Sex	No. of insects	Size			Shape		
			Side	Side $\times$ individual	Measurement error	Side	Side $\times$ individual	Measurement error
Domicile	Female	22	0.891	26.848***	0.294	0.041*	0.021***	0.000
Domicile	Male	25	315.641*	48.289***	0.455	0.049	0.034***	0.001
Goat corral	Female	25	5.431	94.800***	1.301	0.058*	0.028***	0.002
Goat corral	Male	21	3.109	28.339***	2.503	0.032*	0.024***	0.003
Pig corral	Female	14	65.142	27.514***	1.056	0.036	0.024***	0.001
Pig corral	Male	21	0.144	29.281***	0.445	0.059	0.043***	0.001
Storeroom	Female	14	65.142	27.514***	1.056	0.036	0.024***	0.001
Storeroom	Male	16	14.025	43.554***	0.360	0.084	0.056***	0.001

\*  $p < 0.05$ .

\*\*\*  $p < 0.001$ .

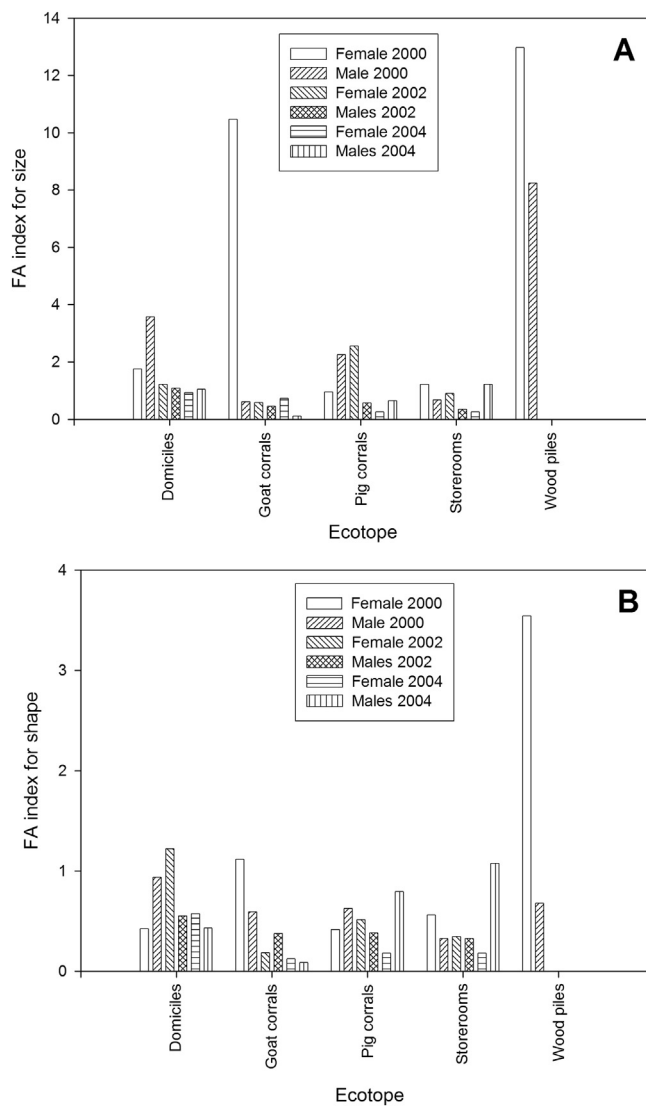


Fig. 4. Fluctuating asymmetry index for wing size and wing shape of female and male *Triatoma infestans* for each collection year (2000, 2002 and 2004) and ecotope. The index is the mean square (MS) for the side-individual interaction effect as shown in the ANOVA output of Tables 1 to 3 corrected with the measurement error (MS/ME). A. Fluctuating asymmetry index for wing size. B. Fluctuating asymmetry index for wing shape.

triatomine populations were smaller, with nearly 30% of sites in late summer or early autumn having only adult triatomines (i.e., recent invasions, not colonizations). *T. infestans* populations subjected to recurrent insecticide spraying are expected to suffer large disturbances and repeated bottleneck events, and thus are susceptible to local differentiation (e.g., Pérez de Rosas et al., 2007). Moreover, pyrethroids exert excito-repellent effects (Diotaiuti et al., 2000) and may promote the active dispersal of triatomines that do not pick up a lethal dose and invasion of other neighboring sites (Gurevitz et al., 2013). Second, pyrethroid insecticides may exert direct effects on *T. infestans* wing development, as shown in insect populations exposed to pesticides (Beasley et al., 2013). The community-based insecticide spraying program in Amamá may have affected wing development and symmetry, especially because the egg-to-adult development time of *T. infestans* averages six months (Rabinovich, 1972) and the residual effects of pyrethroids last for several months indoors (Rojas de Arias et al., 2003).

Our results show that triatomines from recently infested goat corrals had more FA in wing size than those from persistently infested goat corrals, supporting our hypothesis. This suggests that recent in-migrants might have come from other habitats under large environmental stress

Table 5

Linear correlation coefficients between wing size and wing shape individual FA scores for each ecotope, sex and collection year for *T. infestans* from Amamá and neighboring villages.

Year	Ecotope	Sex	Correlation coefficient	
2000	Domicile	Female	0.04	
	Domicile	Male	0.14	
	Goat corral	Female	-0.31	
	Goat corral	Male	0.23	
	Pig corral	Female	-0.63**	
	Pig corral	Male	-0.53**	
	Storeroom	Female	0.10	
	Storeroom	Male	0.21	
	Wood pile	Female	0.06	
	Wood pile	Male	0.11	
2002	Domicile	Female	-0.82**	
	Domicile	Male	-0.62	
	Goat corral	Female	0.20	
	Goat corral	Male	0.02	
	Pig corral	Female	0.17	
	Pig corral	Male	-0.41	
	Storeroom	Female	-0.82**	
	Storeroom	Male	-0.07	
	2004	Domicile	Female	0.31
		Domicile	Male	-0.53**
Goat corral		Female	0.03	
Goat corral		Male	-0.24	
Pig corral		Female	0.11	
Pig corral		Male	-0.01	
Storeroom		Female	0.11	
Storeroom		Male	-0.55	

\*\* p < 0.01.

(e.g., diminishing host-blood sources, large bug density, degree of isolation, and insecticide treatment), such as wood piles. This environmental stress may be reflected in greater levels of FA relative to triatomines from established populations. Any of these factors or a combination of them are expected to trigger flight initiation and eventual invasion of other suitable sites.

The follow-up of triatomine populations from three goat corrals and a pig corral over a four-year period showed that FA patterns varied over time and space, and the individual amounts of FA differed from those displayed by the aggregate sample across all study sites. These patterns are most likely linked to bug movement and the process of house reinfestation with *T. infestans* after insecticide spraying. The shared features among the study corrals was that males appeared before females; females probably reinfested these corrals from other nearby infested sites, and wing FA declined over subsequent years.

Our study is observational and therefore cannot identify the exact factors underlying the FA patterns recorded, which demands an experimental approach. The only effort in this direction assessed the effects of vector density and blood-feeding frequency on wing size and wing shape FA in various species of *Rhodnius*, and established that such effects were sex-dependent (Márquez and Saldamando-Benjumea, 2013). This limitation does not undermine the fact that wings seem to be an appropriate symmetric module to assess habitat-related developmental instability in *T. infestans*.

## 5. Conclusions

This study provides evidence for temporal variation in wing size and shape developmental instability congruent with habitat/host combinations and/or timing of flight dispersal. The history of insecticide spraying, either through direct effects of pyrethroid exposures on insect



development or indirect effects related to flight dispersal and house invasion dynamics, appears to be associated with the FA patterns observed. Whether wing FA, triatomine flight dispersal and house reinfestation are causally linked and FA may provide another marker to identify the sources of reinfestants require further investigation.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meegid.2017.11.012>.

Year	Ecotope	Sex	Correlation coefficient
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	Domicile	Male	− 0.62
	Goat corral	Female	0.20
	Goat corral	Male	0.02
	Pig corral	Female	0.17
	Pig corral	Male	− 0.41
	Storeroom	Female	− 0.82**
	Storeroom	Male	− 0.07
2004	Domicile	Female	0.31
	Domicile	Male	− 0.53**
	Goat corral	Female	0.03
	Goat corral	Male	− 0.24
	Pig corral	Female	0.11
	Pig corral	Male	− 0.01
	Storeroom	Female	0.11
	Storeroom	Male	− 0.55

\*\*  $p < 0.01$ .

### Acknowledgments

We thank Judith Schachter-Broide, Gonzalo M. Vazquez-Prokopec and Leonardo A. Ceballos for processing the insects used in this study. Jean-Pierre Dujardin made valuable suggestions on an early version of this manuscript and Lucia Babino help us in statistical analysis.

### Funding

This work was supported by University of Buenos Aires [20020100100944], CONICET [PIP 11220110101146 and PIP 11220150100846CO], Fundación Bunge & Born and Agencia Nacional de Promoción Científica y Tecnológica from Argentina [PICT 2013-1670]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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