Infection, Genetics and Evolution 74 (2019) 103925

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Research paper

# Fluctuating asymmetry and exposure to pyrethroid insecticides in *Triatoma infestans* populations in northeastern Argentina

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ARTICLE INFO

*Keywords:*
*Triatoma infestans*
Fluctuating asymmetry
Flight dispersal
Pyrethroid resistance
Reinfestation
Wing size
Wing shape

ABSTRACT

Fluctuating asymmetry (FA), a phenotypic marker used as indicator of developmental stress or instability, is sometimes associated with insecticide application and resistance. Here we investigated the occurrence and amount of wing size and wing shape FA in *Triatoma infestans* females and males collected before and 4 months after a community-wide pyrethroid spraying campaign in a well-defined rural area of Pampa del Indio, Argentina. Moderate levels of pyrethroid resistance were previously confirmed for this area, and postspraying house infestation was mainly attributed to this condition. In the absence of insecticide-based selective pressures over the previous 12 years, we hypothesized that 1- if postspraying triatomines were mostly survivors to insecticide spraying (pyrethroid resistant), they would have higher levels of FA than prespraying triatomines. 2- if postspraying triatomines have a selective advantage, they would have lower FA levels than their prespraying counterparts, whereas if postspraying infestation was positively associated with immigrants not exposed to the insecticide, prespraying and postspraying triatomines would display similar FA levels. For 243 adult *T. infestans* collected at identified sites before insecticide spraying and 112 collected 4 months postspraying, wing size and wing shape asymmetry was estimated from landmark configurations of left and right sides of each individual. At population level, wing size and shape FA significantly decreased in both females and males after spraying. Males displayed greater wing size and shape FA than females. However, at a single peridomestic site that was persistently infested after spraying, FA declined similarly in females whereas the reverse pattern occurred in males. Our results suggest differential survival of adults with more symmetric wings. This pattern may be related to a selective advantage of survivors to insecticide spraying, which may be mediated or not by their pyrethroid-resistant status or to lower triatomine densities after insecticide spraying and the concomitant increase in feeding success.

## 1. Introduction

Symmetry of biological structures can be defined as the repetition of parts in different positions and orientations to each other. Such symmetry is a fundamental feature of the body plans of most organisms, expressed in a particular way under the control of a particular genome. Asymmetry is simply a deviation from symmetry (Klingenberg, 2015; Palmer and Strobeck, 1986). Fluctuating asymmetry (FA) is a phenotypic marker that denotes small differences between the left and right sides of a bilateral structure; such differences have a normal distribution around a mean of zero (Palmer and Strobeck, 1986). FA has been considered an indicator of increased developmental instability or reduced developmental homeostasis at the population level (Clarke and McKenzie, 1992; Markow, 1995). Phenotypic selection acting on a population affect the phenotypic variance of traits, including FA (Møller and Swaddle, 1997). In a wide range of animal species, the individuals' asymmetry is negatively correlated with fitness components such as fecundity, growth, and survival (Watson and Thornhill, 1994; reviewed by Møller, 1997; Møller and Swaddle, 1997).

In insect populations, FA has been used as an indicator of environmental stressors such as extreme variations in temperature, starvation, chemical pollution and insecticide application (Beasley et al., 2013). The environmental stress imposed by insecticide applications in genetically variable populations may select for certain alleles that

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https://doi.org/10.1016/j.meegid.2019.103925
Received 11 March 2019; Received in revised form 25 May 2019

ameliorate the immediate stress, but which may also disturb developmental processes and lead to an increased asymmetry (McKenzie and Clarke, 1988; McKenzie and O'Farrell, 1993). For example, resistant strains of the mosquito *Culex quinquefasciatus* (Mpho et al., 2001) and the moth *Heliothis virescens* (Gulzar and Wright, 2014) exhibited increasing levels of FA compared with susceptible strains. Subsequent selection for background modifier genes ameliorated the negative impacts of resistance genes, reducing asymmetry scores to those levels present in susceptible genotypes (McKenzie and O'Farrell, 1993). In the Australian sheep blowfly *Lucilia cuprina*, resistant strains exhibited reduced levels of FA compared with susceptible strains when the former were under long-term selection pressures exerted by insecticide application (Clarke et al., 2000; McKenzie and Clarke, 1988). However, in the absence of such selection pressures, FA increased in resistant individuals of *L. cuprina* (Clarke et al., 2000; McKenzie and Batterham, 1994). To explain these patterns, it was proposed that the initial introduction of the resistant allele resulted in increased developmental "noise" and hence asymmetry; the subsequent selection of a resistant allele re-established normal developmental processes and led to a concomitant decrease in asymmetry (Clarke and McKenzie, 1987; McKenzie and Clarke, 1988; McKenzie and Game, 1987).

*Triatoma infestans* (Hemiptera: Reduviidae, Triatominae) remains as the main vector of Chagas disease in the Southern Cone of South America (WHO, 2017). Vector control campaigns based on house spraying with pyrethroid insecticides reduced the species' distribution range and infestation levels, which led to the interruption of parasite transmission in several regions (WHO, 2017). However, *T. infestans* populations persist in the Gran Chaco ecoregion of Argentina, Bolivia and Paraguay despite recurrent vector control campaigns conducted over several decades (Gürtler, 2009; WHO, 2017). Vector persistence has usually been associated with poor housing conditions, technical shortcomings during insecticide application, active dispersal of bugs from residual or untreated foci, and/or presence of pyrethroid resistance (Germano et al., 2012; Gurevitz et al., 2013; Gürtler et al., 2007; Picollo et al., 2005; Rojas de Arias et al., 2012). Pyrethroid resistance in *T. infestans* emerged in northwestern Argentina and Bolivia by the late 1990s (Picollo et al., 2005; Santo Orihuela et al., 2008). Several foci have subsequently been detected in the Argentine Chaco (Mougabure-Cueto and Picollo, 2015).

Wing FA in *T. infestans* populations was associated with sex, habitat, host-feeding sources, season/year, and the dynamics of house invasion after insecticide applications as promoters of developmental instability (Nattero et al., 2015; Nattero et al., 2017). Adult *T. infestans* from domiciles and specific peridomestic habitats exhibited different degrees of FA depending on sex and season. Female *T. infestans* from wood piles, goat corrals or domiciles in summer and early autumn, and males from domiciles or storerooms exhibited the highest amount of wing FA, suggesting sex-specific asymmetries in flight dispersal potential (Nattero et al., 2017).

As part of a longitudinal program on the eco-epidemiology and control of Chagas disease in northeastern Argentina, virtually all houses in a well-defined rural section of Pampa del Indio municipality were inspected for infestation and sprayed with pyrethroid insecticides (Gurevitz et al., 2011; Gurevitz et al., 2012). Follow-up surveys detected persistent infestations in 23.1% of the houses at 4 months postspraying (MPS), and laboratory bioassays showed that 11 of the 14 tested *T. infestans* populations had reduced susceptibility to deltamethrin (Gurevitz et al., 2012). The RR estimated was 7.17 (Gurevitz et al., 2012). In addition, multiple high-resistant populations of *T. infestans* were reported at 35 km of Pampa del Indio and beyond (Fronza et al., 2016). Using wing geometric morphometry and microsatellite markers, *T. infestans* collected at 4 and 8 MPS were mainly assigned to the house where they had been collected or to a neighboring source (Gaspe et al., 2013; Piccinali et al., 2018). Taken together, these results suggested that postspraying house infestation was mainly generated by pyrethroid-resistant survivors (Gurevitz et al., 2012; Gaspe et al., 2013; Gurevitz et al., 2013; Piccinali et al., 2018).

Here we investigated the occurrence and amount of wing size and wing shape FA in *T. infestans* adults collected in domestic and peridomestic habitats before and at 4 MPS in this rural section of Pampa del Indio. We also investigated the occurrence and amount of wing size and shape FA in a single peridomestic structure that displayed high bug density before and after insecticide spraying (Gurevitz et al., 2012). Various sources of uncertainty led us to formulate three hypotheses. First, we hypothesized that if postspraying triatomines were mostly pyrethroid-resistant survivors to insecticide spraying, they would have higher levels of FA than prespraying populations. This hypothesis was based on the local absence of insecticide-based selective pressures over the previous decade; the low to moderate local levels of pyrethroid resistance, and the model proposed for *L. cuprina* (in which the time that resistant populations were exposed to insecticides determined FA levels).

Our second alternative hypothesis was based on previous results showing that more symmetric individuals are usually associated with higher fitness (Møller, 1997). Postpraying triatomines should have a selective advantage that conferred them greater survival after exposure to pyrethroids, regardless of their resistance status. If postspraying triatomines have a selective advantage, they would have lower levels of FA than prespraying populations.

Persistent foci of *T. infestans* may have other causes (e.g., inadequate insecticide applications, problems with the insecticide itself, and immigration from untreated foci). If this third hypothesis is correct, postspraying infestations originated from triatomines not exposed to the insecticide and they would display levels of FA indistinguishable from those displayed by prespraying populations.

## 2. Materials and methods

### 2.1. Study site and insects

The house infestation survey was conducted in a well-defined rural section of Pampa del Indio Municipality (Chaco province) (25°55′S, 56°58′W) (Fig. 1b,c).The rural area of the municipality was divided in four areas (I-IV) based on infestation levels and logistic reasons. The current study was conducted in a section of Area I (Fig. 1b), which encompassed 353 houses and 37 public buildings in 13 neighboring rural villages as of 2007 (Gurevitz et al., 2011). The district is located in the transition between the dry and humid Argentine Chaco. Houses were composed of human habitations (domiciles) and one or more peridomestic structures for domestic animals and other usage (usually storerooms, kitchens, one or more sites for chickens and other poultry, and one or more corrals).

Demographic and vector surveys conducted in September–November 2007 (prespraying or baseline) showed that *T. infestans* infested 147 (39.8%) house compounds as determined by timed-manual searches with a dislodging aerosol (Gurevitz et al., 2011). Vector control personnel sprayed all sites from every house with suspension concentrate deltamethrin (K-Othrin, Bayer) using a standard dose (25 mg/m$^2$) in December 2007; 34 (23.1%) of houses remained infested at 4 MPS (Gurevitz et al., 2013). All collected triatomines were transported to the laboratory and processed as described elsewhere (Cecere et al., 2002).

The spatial structuring of baseline *T. infestans* populations of Area I showed the existence of two close spatial groups (Western and Eastern) as determined by wing geometric morphometry (Gaspe et al., 2012) (Fig. 1a, Table 1). These two groups were used as reference groups to identify the putative origin of reinfestants (Gaspe et al., 2013). In the current study, wing size and shape FA were analyzed separately for the two groups, which are here denominated Group 1 (Western) and Group 2 (Eastern) (Fig. 1a).

The sample size for each vector survey is presented in Table 1. The prespraying sample, the same used previously for the spatial structuring

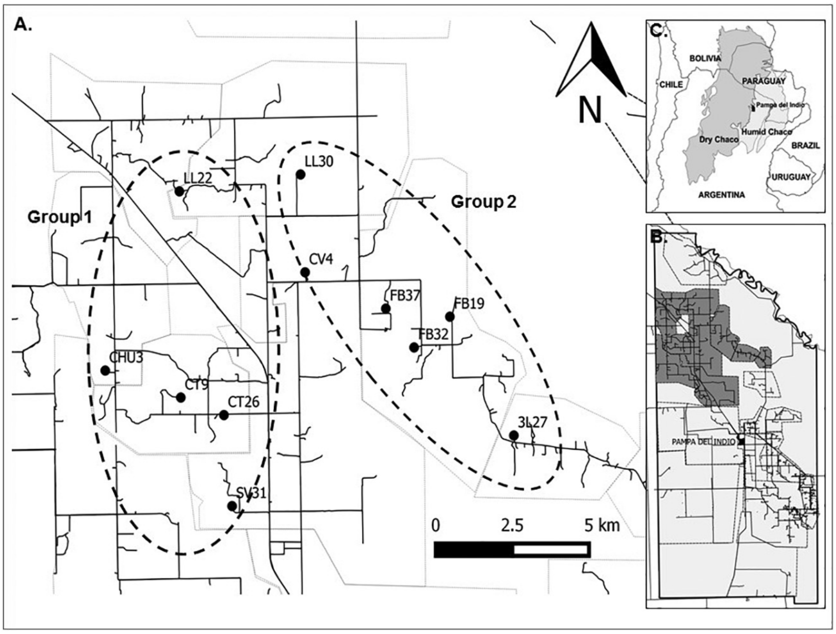

**Fig. 1.** Map of the study area including the location of house compounds and the two reference groups (dashed circles) previously described by Gaspe et al. (2012) in Pampa del Indio, Argentina. Western group: Group 1; eastern group: Group 2. B. Location of Area 1 (dark grey) in the Municipality of Pampa del Indio. C. Location of the Municipality of Pampa del Indio in South America.

study (Gaspe et al., 2012), included 97 females and 146 males collected in domestic and peridomestic sites from 11 houses: five from Group 1 and six from Group 2 (Table 1). Before insecticide spraying, only sites with at least 10 triatomines of the same sex were included in FA measurements, and no domicile with enough females or males was recorded in Group 2 houses; therefore, only triatomines from peridomestic habitats were examined (Table 1). Postspraying triatomines collected at 4 MPS from 35 houses (n = 112) were pooled across sites from the same group owing to small sample sizes (Table 1).

A peridomestic site at house CT9 showed a high-density infestation that persisted between 0 and 4 MPS; this site was examined in detail (Fig. 1a). This site was a small peridomestic structure (1.5 × 1 × 2 m high) with a thatched roof and mud walls (Fig. 1a in Gurevitz et al., 2012). Formerly used as a small chapel, it turned into a nesting or resting site for chickens and ducks. In particular, the sample of this peridomestic structure included 17 females and 32 males collected at baseline, and 14 females and 22 males collected at 4 MPS. These individuals were partially included in the population-level analyses, and were also analyzed separately.

### 2.2. *Wing data collection and asymmetry assessment*

Left and right wings from females and males collected before and after the insecticide spraying campaign were mounted between microscope slides and cover slips, and photographed with a digital camera (Sony MVC-CD300, US) and a stereo-microscope (Zeiss SV11, Germany) using a 6× magnification. For assessing wing size and shape asymmetry, we used landmark-based geometric morphometry. We collected 10 type-I landmarks positioned at vein intersection as described elsewhere (Schachter-Broide et al., 2004) using TPSdig 2.30 software (Rohlf, 2017).

For the assessment of wing size asymmetry, centroid size (CS) was estimated 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 between left and right sides was done with a Procrustes approach by computing differences of landmark coordinates after a Procrustes superimposition of the configurations from both sides.

**Table 1**
Number of *T. infestans* females and males included in this study. Timing of vector survey (prespraying, postspraying), reference groups, houses and ecotopes (domiciles, peridomiciles). The house ID includes the community (letters) and the house number. Reference groups were derived from a geometric morphometry-based spatial structuring study.

| Timing of vector survey | Reference groups | House ID | Ecotopes | Number of females | Number of males |
|---|---|---|---|---|---|
| Prespraying | Group 1 | CHU03 | Domicile | 10 | 13 |
| | | LL22 | Domicile | 14 | 14 |
| | | SV31 | Peridomicile (chicken coop) | 11 | 12 |
| | | CT09 | Peridomicile (domestic chapel) | 13 | 14 |
| | | CT26 | Peridomicile (storeroom) | 15 | 15 |
| | Group 2 | FB19 | Peridomicile (chicken coop) | 0 | 15 |
| | | FB32 | Peridomicile (chicken coop) | 0 | 15 |
| | | FB37 | Peridomicile (chicken coop) | 10 | 11 |
| | | LL30 | Peridomicile (kitchen) | 0 | 11 |
| | | 3L27 | Peridomicile (chicken coop) | 14 | 14 |
| | | CV04 | Peridomicile (pig corral) | 10 | 12 |
| Postspraying | Group 1 | | Domicile | 11 | 7 |
| | | | Peridomicile | 13 | 35 |
| | Group 2 | | Domicile | 8 | 6 |
| | | | Peridomicile | 20 | 12 |
| Total | | | | 149 | 206 |

All left wings were first reflected to their mirror images. Separate landmark configurations were digitalized twice in both wings of each individual triatomine to estimate measurement error (Palmer, 1994). Configurations for each sex within each geographic group and for the individual peridomestic site, before and after insecticide spraying, were superimposed using the least-squares Procrustes method (Klingenberg and McIntyre, 1998).

Asymmetry can be partitioned into directional and non-directional components depending on the population distribution of left-right differences; both have specific statistical properties, distinct biological origins and implications (Klingenberg, 2015). Directional asymmetry (DA) occurs whenever a trait consistently develops in different manners on the left and right body sides. DA is presumably unrelated to developmental stability (Palmer, 1994). Non-directional asymmetry measures FA. Two-way mixed ANOVAs were used to assess the occurrence of DA and FA on wing size for females and males collected before spraying and at 4 MPS. Wing side was used as a fixed factor and individual as a random factor (Palmer and Strobeck, 1986).

The corrected FA indices (FAI) for size were estimated as the mean square (MS) of the interaction between side and individual of the two-way ANOVAs corrected for measurement error (ME) in all cases. These indices measure the intensity of FA for each reference group. Increasing values of FAI are interpreted as increasing values of FA.

Procrustes ANOVAs were performed in the same relevant groups as for size 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 corrected FAI for shape was estimated as the mean square (MS) of the interaction between side and individual of the Procrustes ANOVAs corrected for measurement errors (MS x $10^3$/ME x $10^3$).

To analyze allometry we computed the linear correlation coefficients between individual FA scores for size and for shape (Procrustes distances) for each reference group. Morphometric and statistical analyses were done using MorphoJ 1.05f (Klingenberg, 2011), the CLIC 99 package (http://xyom-clic.eu/the-clic-package/) and InfoStat 2018 (Di Rienzo et al., 2018).

## 3. Results

### 3.1. Group-specific fluctuating asymmetry before and after insecticide spraying

In total, including prespraying and postspraying surveys, 355 adult *T. infestans* were examined by wing geometric morphometry (Table 1). Descriptive statistics of wing size asymmetry for each study group showed that 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 (Supplementary material: Table S1). The signed size differences between right and left wings were negative in 7 groups and positive in 7 groups. Skewness varied between −0.64 and 0.42 and kurtosis between −1.41 and 0.12 (Supplementary material: Table S1). Prespraying females and males exhibited significantly higher wing size FAI than their postspraying counterparts collected in domiciles and peridomiciles of both geographic groups (Table 2, Fig. 2). Before insecticide spraying, FAI varied between 239 and 431 in domiciles and between 249 and 682 in peridomiciles of both groups (MS side x individual, Table 2). Postspraying FAI varied between 158 and 196 in domiciles, and between 99 and 240 in peridomiciles. Prespraying peridomestic males of Group 1 exhibited the highest FAI (Fig. 2). Males displayed higher FAI than females regardless of insecticide exposure (prespraying versus postspraying) and habitat (domiciles versus peridomiciles), except for prespraying triatomines of Group 2, which showed the reversed pattern (Fig. 2).

Procrustes ANOVA showed a significant occurrence of wing shape FA for all occasions (Table 2). Prespraying females and males exhibited higher FAI than their postspraying counterparts both in domiciles and peridomiciles (Fig. 3). The wing shape FAI of domestic triatomines varied between 76 and 132 (prespraying), and between 37 and 56 (postspraying) (MS side x individual, Table 2). For peridomestic bugs, wing shape FAI varied between 80 and 152 (prespraying) and between 47 and 87 (postspraying). Males exhibited higher FAI than females in both habitats and vector surveys, except for postspraying domiciles from Group 1 and prespraying peridomiciles from Group 2. Regarding the allometric analysis, the linear correlation coefficients between individual FA scores for wing size and wing shape were not statistically significant for any of the groups (Table 3).

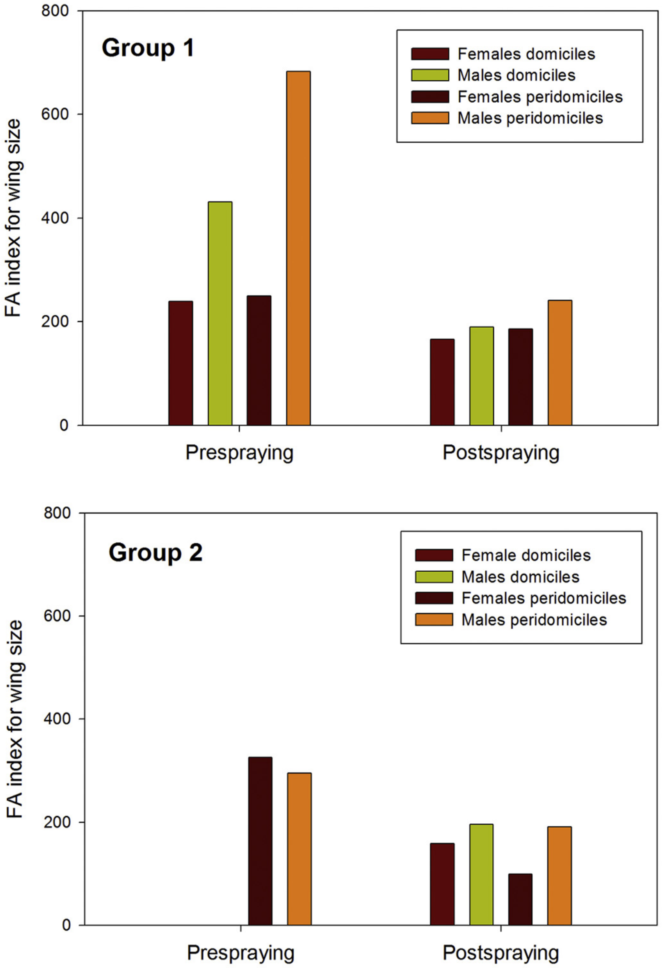

**Fig. 2.** Fluctuating asymmetry index for wing size for prespraying and postspraying *T. infestans* females and males collected in domiciles and peridomiciles from the two reference groups in Pampa del Indio, Argentina. FA indices represent the mean square (MS) of the side-individual interaction effect corrected for measurement error (MS/ME) as shown in the ANOVA output of Table 2.

### 3.2. Fluctuating asymmetry in a persistently infested peridomestic site

Wing size FA was statistically significant in prespraying and postspraying females and males (Table 4). Wing size FAI varied from about 30 in postspraying females to 1036 in postspraying males (Fig. 4a). Females showed lower FAI than males before and after insecticide spraying (Fig. 4a). Prespraying females showed higher FAI than postspraying females, whereas the reverse occurred in males (Fig. 4a).

The Procrustes ANOVA showed significant FA for females and males in both surveys (Table 4). Wing shape FAI varied from 1 to 5 (Fig. 4b). Postspraying females and males exhibited the lowest and highest FAIs, respectively (Fig. 4a). When comparing females and males in relation to insecticide spraying, prespraying females exhibited higher FAI than

**Table 2**

Two-way mixed and Procrustes ANOVAs to partition directional mean square (MS) (MS side) ($\times 10^3$ for shape) and non-directional (MS side $*$ individual) ($\times 10^3$ for shape) asymmetry from wings of *T. infestans*. Groups were derived from a geometric morphometry-based spatial structuring study. ME = measurement error.

| Group | Timing of vector survey | Sex | Habitat | No. of insects | Size |  |  | Shape ($\times 10^3$) |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  | MS side | MS side × individual | ME | MS side | MS side × individual | ME |
| 1 | Prespraying | Female | Domicile | 24 | 351.330* | 74.645*** | 0.312 | 0.066 | 0.048*** | 0.001 |
|  |  |  | Peridomicile | 39 | 300.502* | 57.207*** | 0.229 | 0.146*** | 0.042*** | 0.001 |
|  |  | Male | Domicile | 27 | 168.373 | 52.519*** | 0.122 | 0.101 | 0.039*** | 0.000 |
|  |  |  | Peridomicile | 41 | 325.730 | 92.543*** | 0.136 | 0.248*** | 0.056*** | 0.004 |
|  | Postspraying | Female | Domicile | 11 | 275.887* | 41.347*** | 0.249 | 0.094*** | 0.031*** | 0.001 |
|  |  |  | Peridomicile | 13 | 8.822 | 110.860*** | 0.595 | 0.103** | 0.042*** | 0.001 |
|  |  | Male | Domicile | 7 | 25.360 | 48.220*** | 0.254 | 0.030 | 0.027*** | 0.001 |
|  |  |  | Peridomicile | 35 | 1.660 | 64.940*** | 0.269 | 0.133** | 0.049*** | 0.001 |
| 2 | Prespraying | Female | Peridomicile | 34 | 84.702 | 93.320*** | 0.287 | 0.080* | 0.063*** | 0.000 |
|  |  | Male | Peridomicile | 78 | 314.485* | 76.052*** | 0.258 | 0.090** | 0.056*** | 0.001 |
|  | Postspraying | Female | Domicile | 8 | 117.933 | 45.633*** | 0.287 | 0.048* | 0.023*** | 0.001 |
|  |  |  | Peridomicile | 20 | 14.240 | 35.137*** | 0.353 | 0.099 | 0.038*** | 0.001 |
|  |  | Male | Domicile | 6 | 113.059 | 48.423*** | 0.247 | 0.018 | 0.024*** | 0.001 |
|  |  |  | Peridomicile | 12 | 250.576 | 54.103*** | 0.283 | 0.041 | 0.031*** | 0.001 |

* $p < .05$.

** $p < .01$

*** $p < .001$.

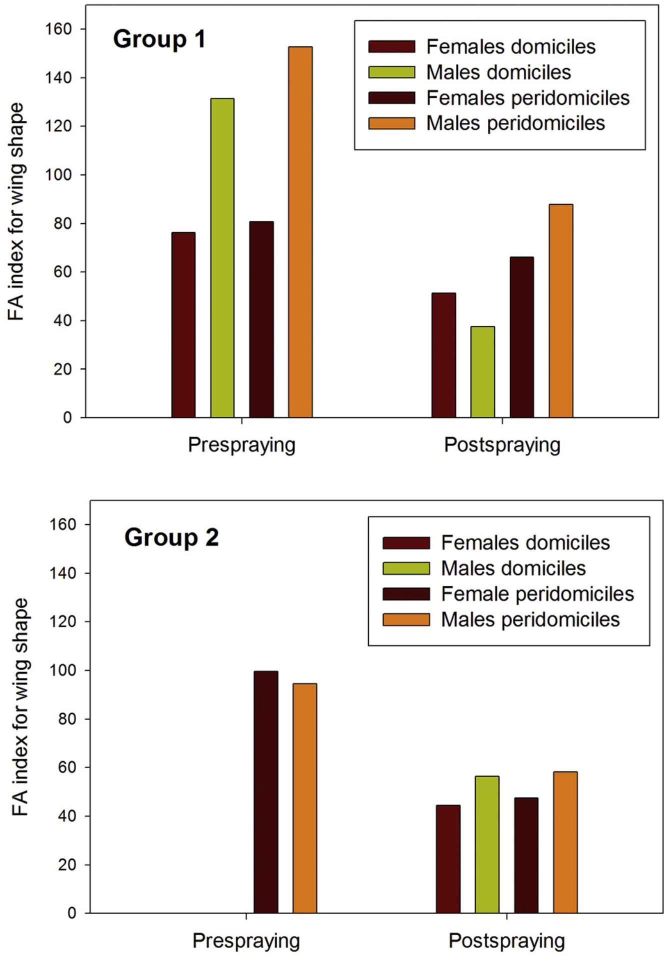

**Fig. 3.** Fluctuating asymmetry index for wing shape for prespraying and postspraying *T. infestans* females and males collected in domiciles and peridomiciles for the two reference groups in Pampa del Indio, Argentina. FA indices represent the mean square (MS) of the side-individual interaction effect corrected for measurement error (MS/ME) as shown in the ANOVA output of Table 2.

**Table 3**

Correlation coefficients between wing size and wing shape individual FA scores for female and males of *T. infestans* from each study group. Groups 1 and 2 were derived from a geometric morphometry-based spatial structuring study.

| Group | Timing of vector survey | Sex | Habitat | Correlation coefficient |
|---|---|---|---|---|
| 1 | Prespraying | Female | Domicile | −0.039 |
|  |  |  | Peridomicile | −0.138 |
|  |  | Male | Domicile | −0.146 |
|  |  |  | Peridomicile | −0.145 |
|  | Postspraying | Female | Domicile | −0.021 |
|  |  |  | Peridomicile | 0.411 |
|  |  | Male | Domicile | −0.024 |
|  |  |  | Peridomicile | −0.188 |
| 2 | Prespraying | Female | Peridomicile | 0.365 |
|  |  | Male | Peridomicile | −0.151 |
|  | Postspraying | Female | Domicile | 0.088 |
|  |  |  | Peridomicile | 0.262 |
|  |  | Male | Domicile | 0.009 |
|  |  |  | Peridomicile | 0.098 |

postspraying females, whereas males displayed the reverse pattern (Fig. 4a and b). For allometric analysis, the correlation coefficients were not statistically significant for any of the reference groups (Supplementary material: Table S2).

## 4. Discussion

Our study documents that wing size and wing shape FA consistently decreased in domestic and peridomestic *T. infestans* of both sexes shortly after a community-wide spraying campaign with pyrethroid insecticide. Males exhibited significantly larger levels of FA than females in most of the populations examined. At the level of a persistently infested peridomestic site, the patterns of variation of FAI for both wing size and shape were congruent with the general outcome for females (i.e., reduced FAI after spraying), whereas males displayed the reverse pattern.

We found more symmetric wings (lower FA) in postspraying females and males than in their prespraying counterparts, supporting our second hypothesis, i.e., postspraying triatomines have a selective advantage over their prespraying counteparts. The decrease in FA for wing size and shape at 4 MPS was consistent in both geographic groups. Considering the local occurrence of moderate levels of pyrethroid resistance and the absence of community-wide insecticide applications

**Table 4**

Two-way mixed and Procrustes ANOVAs to partition directional mean square (MS) (MS side) ($\times 10^3$ for shape) and non-directional (MS side * individual) ($\times 10^3$ for shape) asymmetry from wings of adult *T. infestans* collected in a persistently infested peridomestic site.

| Timing of vector survey | Sex | No. of insects | Size | | | Shape ($\times 10^3$) | | |
|---|---|---|---|---|---|---|---|---|
| | | | MS side | MS side × individual | Measurement error | MS side | MS side × individual | Measurement error |
| Prespraying | Female | 17 | 0.049 | 0.096*** | 0.000 | 0.236 | 0.074*** | 0.002 |
| | Male | 32 | 0.238 | 0.070*** | 0.000 | 0.107* | 0.053*** | 0.001 |
| Postspraying | Female | 14 | 0.014 | 0.080*** | 0.003 | 0.041 | 0.028*** | 0.002 |
| | Male | 22 | 0.189 | 0.059*** | 0.000 | 0.073 | 0.043*** | 0.006 |

* $p < .05$.

*** $p < .001$.

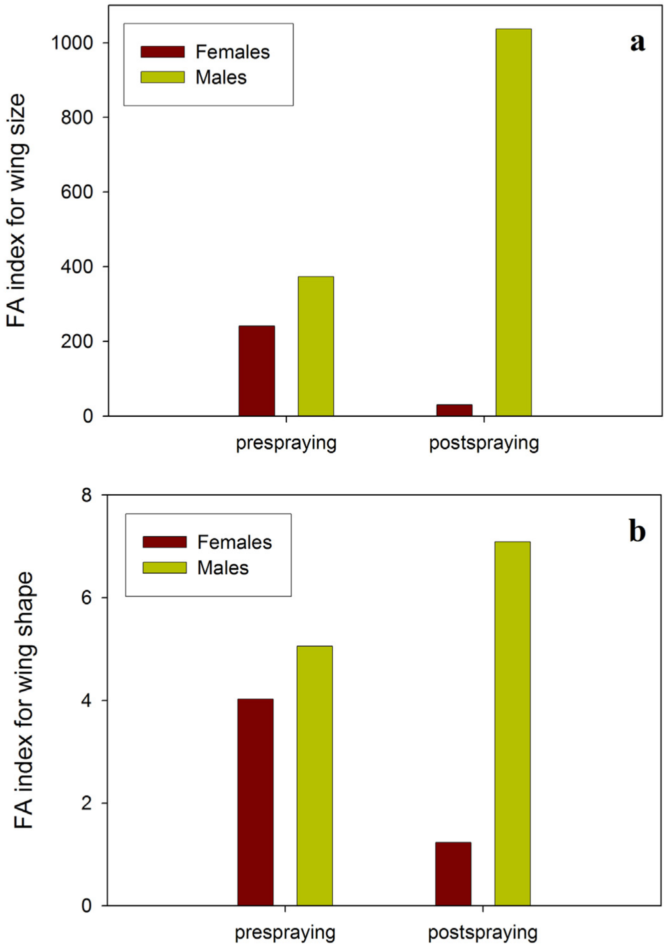

**Fig. 4.** Fluctuating asymmetry index for wing size and wing shape for females and males of *T. infestans* collected in a persistently infested peridomestic site before and after insecticide spraying in Pampa del Indio, Argentina. FA indices represent the mean square (MS) of the side-individual interaction effect corrected for measurement error (MS/ME) as shown in the ANOVA output of Table 4. a. Wing size FA indices. b. Wing shape FA indices.

over the prior 12 years, at least two alternative, not mutually exclusive explanations may account for postspraying triatomines being more symmetric than prespraying insects.

First, the *T. infestans* that survived to insecticide spraying may have a selective advantage mediated or not by the pyrethroid-resistant condition. In other insect species, asymmetrical phenotypes have detrimental consequences on physiological, behavioral and fitness-related traits (Clarke, 1998; Markow, 1995; Møller, 1999). For example, wing asymmetry mechanically compromises flight performance and wing function in insects (Crespi and Vanderkist, 1997; McLachlan, 1997; McLachlan, 2010). Thus, wing symmetry or any other closely correlated trait(s) could confer these individuals the advantage to avoid or reduce their exposure to insecticides through increased dispersal capacities. Considering the context of moderate level of pyrethroid resistance, the proportion of adult triatomines that were resistant at 4 MPS is expected to increase relative to baseline values given their recent exposure to pyrethroids. Most of the sites that remained infested over 0–4 MPS included many late-instar nymphs and adults that most likely were survivors from adults and immatures of the same generation showing moderate levels of pyrethroid resistance (Gurevitz et al., 2012). A possible inference is that pyrethroid-resistant *T. infestans* have more symmetric wings, as recorded in two resistant strains of the maize weevil *Sitophilus zeamais* under repeated insecticide-based selective pressures (Ribeiro et al., 2007).

The effects of insecticide application on wing developmental instability are modified by insect body size in a context of moderate pyrethroid resistance. *Triatoma infestans* female and male adults collected at 4 MPS had significantly larger wings than their prespraying counterparts (Gaspe et al., 2013), whose wing size was positively and highly significantly correlated with total body length (Gaspe et al., 2012), as in other insects (e.g., Kaspari and Weiser, 1999). Moreover, total body length correlated closely with other metrics of body size (e.g., total body weight) for most stages of *T. infestans* in the field, and was associated positively with nymphal bloodmeal contents and female fecundity (Gürtler et al., 2017). Because of the rather long time period required to molt to fifth-instar nymph or to adult from the preceding stage (range, 2–4 months depending on temperature and blood-feeding rates), we infer that a large fraction of adult triatomines at 4 MPS had been fourth- or fifth-instars or adults that survived the insecticidal campaign. The fifth-instar nymphs were close to their adult body size, which is mainly determined at the last immature instar when most of the body mass is accumulated (Nijhout et al., 2014). Fourth- and fifth-instar nymphs of *T. infestans* are the least susceptible to pyrethroid insecticides, especially in pyrethroid-resistant insects, where the lethal dose of insecticide per biomass unit steadily increased with stage (Germano and Picollo, 2018). Likewise, in *Anopheles* mosquitoes, bigger adults were more likely to survive insecticide treatment (Owusu et al., 2017). These findings suggest that the insecticide spraying campaign selected for bigger triatomines, which had or developed more symmetric wings than the non-selected prespraying adult triatomines, and were most likely favored by improved density-dependent blood-feeding success and engorgement following stark reductions in population size.

Second, triatomine relative densities decreased substantially (by 90%) after insecticide spraying (Gurevitz et al., 2013). This implies concomitant increases in density-dependent blood-feeding success and engorgement of survivors, a lower nutritional stress, and development of wings that are more symmetric. This mechanism is supported, at least for wing size, by experimental evidence. There was an increment in wing size FA for *Rhodnius robustus*-like females reared on high feeding frequency and high-population density (Fig. 3, Márquez and Saldamando-Benjumea, 2013).

Sexual differences in wing FA occur in several insect species, with females consistently displaying more symmetric wings than males

(Blanckenhorn et al., 2003; Breuker et al., 2007; Corrêa et al., 2006), as in our study populations. These differences in FA between sexes were related to sexual selection and sex-related flight dispersal capacities (Breuker et al., 2007; Corrêa et al., 2006). In Triatominae, males of the *Triatoma dimidiata* complex showed a 38% greater asymmetry than females (Nouvellet et al., 2011), and in *Panstrongylus megistus* more asymmetric wings were negatively related to flight activity (Soares et al., 1997). Accordingly, our results for *T. infestans* suggest females would have greater flight activity and a longer dispersal range. In various types of experiments, females of *T. infestans* initiated flight more frequently than males (Galvão et al., 2001; Gurevitz et al., 2006; Minoli and Lazzari, 2006). In the field, males showed higher genotypic and phenotypic structure than female populations, also suggesting the latter had a greater dispersal capacity (Gaspe et al., 2012; Marcet et al., 2008; Pérez de Rosas et al., 2013).

The persistently infested peridomestic site was the only one that had a considerable number of postspraying triatomines for a site-level analysis. The reduced FAI in postspraying females matched the general negative trend in wing size and shape FA after community-wide insecticide application. Postspraying males exhibited by far the highest FAI. A more detailed analysis of the individual FA of the 22 males in this group revealed that five of them were highly asymmetric. Excluding these five insects yielded the same general pattern of lower asymmetry in postspraying males. Although the origin of these five individuals is uncertain, one possibility is that they were immigrants. A microsatellite-based analysis of postspraying triatomines from this site indicated that some males were immigrants whereas others most likely survived the spraying. Assignment tests excluded houses located > 9 km from the study area (Piccinali et al., 2018). Geometric morphometry of wing shape also confirmed this pattern (Table 2 in Gaspe et al., 2013).

This observational study cannot identify the causal mechanisms underlying the FA patterns recorded. Experiments that address the effects of pyrethroid application on resistant and susceptible populations of *T. infestans* are required to assess their effects on wing developmental instability. Despite these limitations, wing FA is adequate to investigate population-level patterns of phenotypic variation related to habitat, dispersal and reinfestation dynamics.

## 5. Conclusions

This study provides evidence of a differential occurrence of *T. infestans* adults with more symmetric wings after a round of residual spraying with pyrethroid insecticide. Alternative explanations may account for this pattern: 1- postspraying survivors with more symmetric wings and/or larger body size may have a selective advantage or 2- a decrease in triatomine relative densities after insecticide spraying with the concomitant increases in density-dependent blood-feeding success. The sex-biased wing asymmetry pattern also suggested females would have greater dispersal ability than males. This trait may potentially affect the flight-mediated dynamics of house reinvasion and effectiveness of triatomine control operations in the Gran Chaco ecoregion.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103925.

## Funding

This study was supported by awards from Agencia Nacional de Promoción Científica y Tecnológica (PICT 2014-1952) and CONICET (PIP 11220150100846CO). REG acknowledges with thanks the support of PICT 2014-2113, Fundación Bunge y Born (2015-2018), and the University of Buenos Aires. The funding sources had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Acknowledgements

Juan M. Gurevitz, Leonardo Ceballos, Gustavo Enríquez, Julián Alvarado-Otegui, and Paula Ordoñez-Krasnowski provided field and laboratory assistance. All authors from this work are members of the CONICET Researcher's Career.

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