

Article

Insect Fluctuating Asymmetry: An Example in Bolivian Peridomestic Populations of *Triatoma infestans* (Klug, 1834) (Hemiptera: Reduviidae)

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Abstract: Fluctuating asymmetry (FA) is a morphometric tool used to measure developmental instability in organisms which have been exposed to stress or other adverse conditions. Phenotypic variability in response to stressors are the result of interactions between genomes and the environment, acting in a noisy developmental system. Most of the organisms have bilateral symmetry with a repetition of structures in different positions or orientations; asymmetrical variation has been a morphological response associated with insecticide application inducing disturbances in endocrinal system product of the chemicals. *Triatoma infestans* (is the main vector of Chagas disease in South America. The availability of food sources varies for populations of *T. infestans* living in different habitats; insects that inhabit the intradomicile feed preferentially on human blood, whereas insects that develop in the peridomicile feed on the blood of the other mammals and birds. The following research evaluate the FA to the different ecotopes in two geographical areas of Chuquisaca Bolivia; Yamparáez/Sotomayor of the high inter-Andean valleys and Huacaya/Imbochi of the boreal Chaco and a CIPEIN laboratory strain population. A combination of advanced morphometrics tools and multivariate analysis were used to quantify the levels of asymmetry produced by pyrethroid near to the peridomiciles in Bolivia. Populations from Yamparáez/Sotomayor were found to have higher levels of FA which the combination of environmental conditions such as low temperatures avoid greater permanence in the habitat and more exposition to insecticide. A better understanding of the combination of these tools will allow researchers to implement better public policies to regulate insecticide applications and to understand how certain organisms adapt to multiple stressors.

Keywords: fluctuating asymmetry; pesticides; geometric morphometrics; vector; developmental instability

1. Introduction

Triatoma infestans (Klug, 1834) (Hemiptera: Reduviidae: Triatominae) is one of the main vectors of Chagas disease in South America [1–3]. This hematophagous insect, a synanthropic species, has adapted to the human habitat: domestic and peridomestic areas

(warehouses and pens for domestic animals) [4–6]. According to the World Health Organization, there are 6 million people infected with the parasite *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae), the causal agent of Chagas disease, in Latin America and it is estimated that 75 million people are at risk of contracting the disease. Complications of chronic disease can be cardiac abnormalities and dilatation of the esophagus and colon [7].

Considering the high epidemiological importance of reducing the vector of Chagas disease transmission in South America, the attempts to eliminate the vector insect in the intra and peridomicile areas started to include spraying insecticides in 1991 [8,9]. In Chuquisaca, Bolivia, they started to use insecticides in 2000 in the intra and peridomicile areas with the purpose of eliminating *T. infestans*. In this way, the indices of intradomiciliary infestation in 2017 were less than 3% in the inter-Andean valley area; however, in the Chaco area, they were more than 7% [10,11]. In both geographical areas, the infestation rates in the peridomicile area were higher than 14% [11].

In South American countries such as Bolivia, Argentina and Paraguay, populations of *T. infestans* resistant to pyrethroid insecticides were reported [5,12–16]. Resistance to pyrethroid insecticides in populations of *T. infestans* has been reported in Bolivia using biological test in eggs and nymphs of the first instar [14,16–19]. In the inter-Andean valleys of Chuquisaca, Bolivia, Lardeux et al. carried out studies of *T. infestans* detecting resistance to pyrethroid and sensitivity to organophosphate and carbamate insecticides [17].

The peridomestic habitat of *T. infestans* differ substantially between inter-Andean valleys and Chaco; the building materials, the environmental temperature and the relative humidity have an influence on the microenvironments [20]. The availability of food sources varies for populations of *T. infestans* living in different habitats; insects that inhabit the intradomicile feed preferentially on human blood, whereas insects that develop in the peridomicile feed on the blood of other mammals and birds [10]. In addition, these insects are exposed to different levels of environmental pollution or toxic substances, such as insecticides [21]. Stress in insects sometimes can be associated with insecticide application and their development [22–28]. Fluctuating asymmetry (FA) allows monitoring the stress of organism in the laboratory and natural environments, since genetic and environmental changes can increase FA with changes in developmental homeostasis expressed in adult morphology. These disturbances include extreme temperatures and contact with chemicals, and they tend to increase as habitats become geographically marginal; this includes exposure to chemical toxins [29]. Brouwer et al. [30] highlighted the value of the integration of studies combining biochemical, physiological and ecological approaches in their assessment of developmental instability. Eeva et al. [31] tested the combination of this approaches in two hole-nesting passerines where the foods' exposure to heavy metals has induced high levels of FA, on the other hand, Benítez et al. [26] identify high levels of FA related to the effect of pine resin and the stress of pine plantation into native species of beetles which live in the native understory of the plantation.

FA is a random deviation from bilateral symmetry that is normally distributed around a mean of 0, and it has been widely used to infer developmental instability [32]. FA is considered an approximate measure of environmental and genetic stress [33]. Directional Asymmetry (DA) measures the tendency of a trait to be consistently developed in a different way on the right and left sides of the body, subtle patterns of DA are a phenomenon widespread in animals [34–37].

The factors that influence the development of FA in insects have been widely studied in relation with food and habitat. For example, Benítez et al. [38] conducted a study in *Macaria mirthae* Vargas et al., 2005 (Lepidoptera: Geometridae), a native moth from Chile (north populations) feeding preferentially on the Fabacea *Acacia macracanta* Humb. Et Bonpl. ex Willd. (Leguminosae) species. Due to the loss of its habitat, it moves towards Fabacea *Leucaena leucocephala* (Lamarck) (Leguminosae), showing FA and DA on the left and right wings of *M. mirthae* species; in the specimens, DA was detected in moths that feed on the native plant and FA in the insects of the exotic plant. Nunes et al. [39] carried out a

study in *Apis mellifera* (Linnaeus, 1761) (Hymenoptera: Apidae) from 16 locations in five geographical regions of Brazil, located in areas with low and high impact of environmental disturbance. The authors observed the existence of FA in bees in the shape of wings but not in size, in locations with high levels of environmental pressure.

T. infestans with FA have significant association with the food source, habitat, insecticide spraying cycles and seasons related to the dispersal of adult insects from domestic animal pens to the intradomicile [40]. *Triatoma infestans* that inhabit the intradomicile had lower FA indices compared with specimens of the peridomicile; according to sex, males presented higher FA indices compared with females, males have a greater tendency to the dispersion from one pen to another or the intradomicile [41–43]. FA rates in *T. infestans* wings were higher before insecticide spraying in the peridomicile area compared with FA rates after chemical treatment [40]. Therefore, the aim of this research is to combine the use of two morphological tools, geometric morphometrics and fluctuating asymmetry, to evaluate the influence of insecticides on the levels of developmental instability and also their relationship with the environmental quality in peridomestic habitats in Bolivia.

2. Materials and Methods

2.1. Study Area

The study was conducted in two geographical regions of Chuquisaca, Bolivia: one location in the inter-Andean valley: Yamparáez/Sotomayor (Lat. 19°19' S Long. 65°60' O), high valley, and the other location is Huacaya/Imbochi (Lat. 19°48' S Long. 64°54' O) located in the dry Chaco (Figure 1). According to Navarro and Maldonado [44], the high inter-Andean valleys are found in the Tucumano-Boliviano region, its geographical and environmental characteristics are: altitude more than 2900 m above sea level with high plateau zones, temperatures are around 15 °C and humidity 40% approximately. The dry Chaco region is located at the east of the Eastern Mountain Range, a region of flat arid lands, with temperatures above 30 °C, and low humidity, around 20%, it is called the Bolivian Boreal Chaco region. Both regions are endemic for Chagas disease. In Yamparáez/Sotomayor, adult *T. infestans* were collected from 10 peridomestic habitats, and in Huacaya/Imbochi, adult insects were collected from 13 peridomestic habitats. Populations of *T. infestans* in both regions were handled with pyrethroid insecticides since the year 2000, with both *T. infestans* populations developing a resistance to pyrethroid insecticides once or twice a year [11].

2.2. Insect Sampling and Preparation

In total, 69 adults of *T. infestans* were collected in peridomestic locations (pens and chicken coops). Overall, 28 females and 41 males were distributed as follows: Yamparáez/Sotomayor 14 and 24, Huacaya/Imbochi 14 and 17, females and males, respectively. Between July and August 2018, the capture was carried out for one hour in each pen or chicken coop, using a clamp each individual was introduced in a glass vial and preserved in alcohol (96%) for further analyses. In the laboratory, wings were mounted on slides with Euparal® for further morphometric analyses, and they were photographed with a Celestron Handheld Digital Microscope pro 5 MP.

To compare FA levels of *T. infestans* from two peridomestic environmental (Yamparáez/Sotomayor and Huacaya/Imbochi) we used a control population, reared in our laboratory, standard strain CIPEIN of adult *T. infestans*, donated by Entomology Laboratory of Programa Chagas from Chuquisaca, Bolivia, first generation in our laboratory: 14 females and 14 males. This strain was developed under controlled conditions of temperature 23 °C +/- 2, humidity 50% +/- 2 and 12 h night; 12 h day.

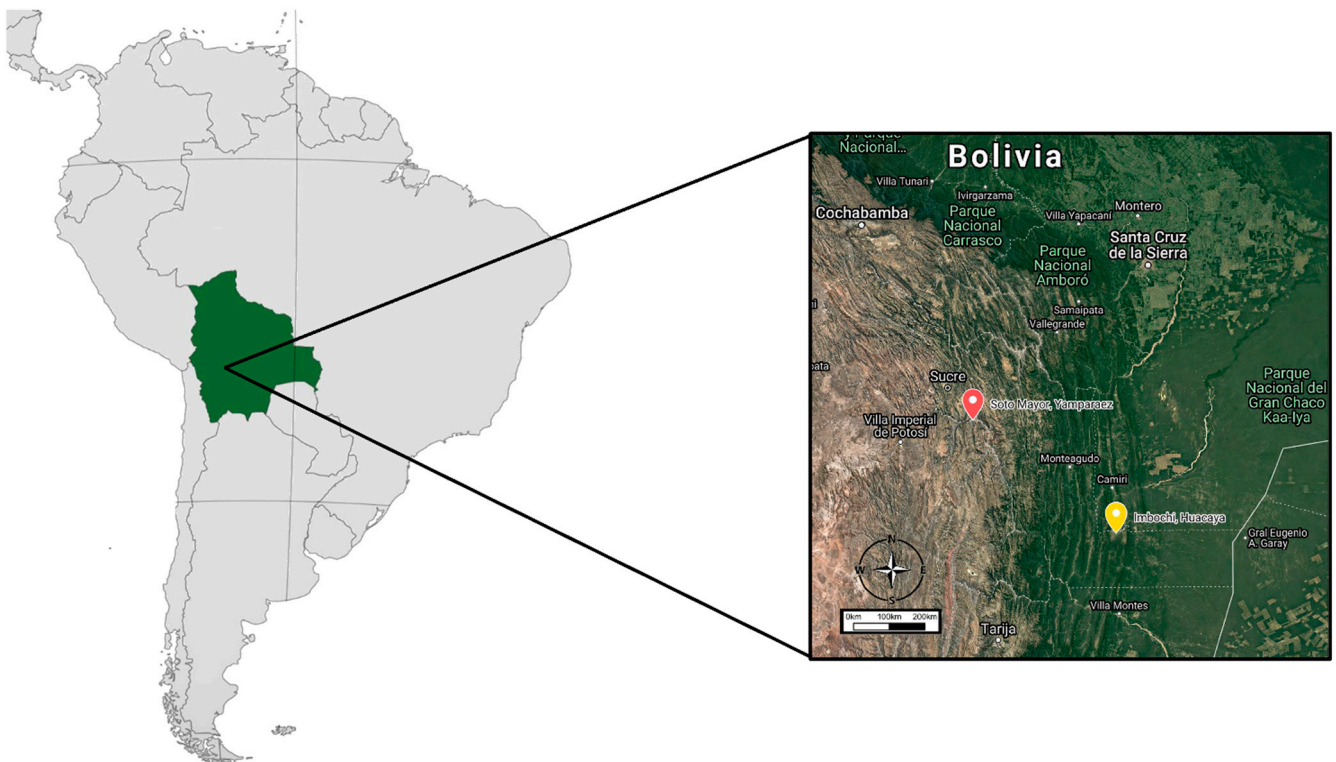


Figure 1. Coordinate information from the Military Institute of Geography for the two locations where *Triatoma infestans* populations were sampled in Chuquisaca: Sotomayor/Yamparáez (red), Huacaya/Imbochi (yellow).

2.3. Shape Analyses

Nine landmarks were digitized, using the software TpsDig 2 V.231 [45], for both right and left wings, according to their external anatomy (Figure 2). The landmarks were aligned applying a Procrustes superimposition method [46]. This procedure removes size, position and orientation information to standardize each specimen based on centroid size. The digitized wings exhibit matching symmetry, which means that the shape analysis included the reflection of all configurations from one body side to its mirror image [36,47]. To calculate the measurement error (ME), the right and left wings were digitized twice [48].

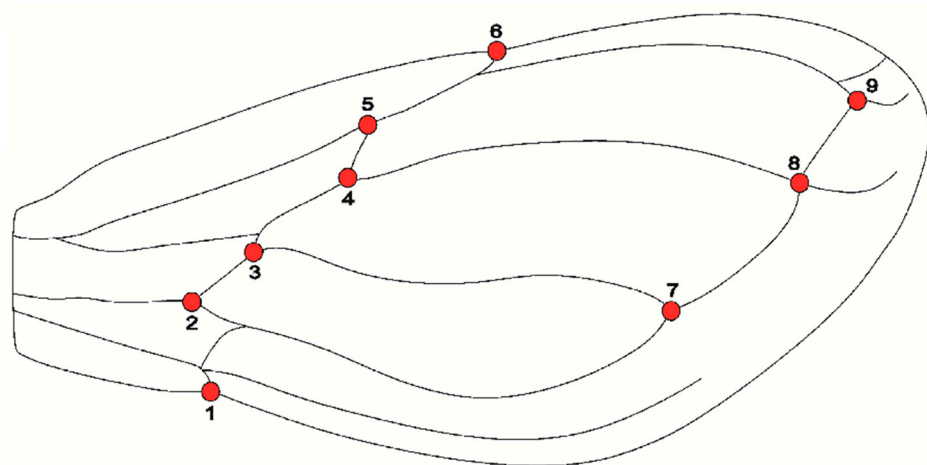


Figure 2. Graphical representation of the wing of *T. infestans* with nine landmarks at the vein intersections.

Procrustes ANOVA is a tool commonly used to analyse asymmetry patterns in morphometric data. The elements of an ANOVA, such as the mean of the squares (MS) and the sum of the squares (SS), which are dimensionless, are essential to evaluate the intensity of the observed asymmetry [36,48]. FA is defined as those random deviation occurring between the left and right sides in a bilateral organism. It is the variation of the individual asymmetry vectors around the means of all the configurations from each side [49].

Following the Protocol of Benítez et al. [27] a comparison between FA intensities was performed by the MS values of shape from a Procrustes ANOVA and a multivariate regression of shape vs. Procrustes FA Scores, using the Procrustes distances of the asymmetry component of the data. Finally, in order to see morphometric differentiation between populations, a Principal component analysis (PCA) was performed with the covariance matrix of wing shape using the software MorphoJ 1.06 d [50].

3. Results

The measurement error was assessed in all the populations studied, in order to avoid any type of error associated with the data. The results of a Procrustes ANOVA indicated that MS values of FA (Ind*side) exceeded the MS values of error, implying that there is no ME in the data (Tables 1–3).

Table 1. Procrustes ANOVA for both centroid size and shape from CIPEIN Population *Triatoma infestans*, (dimensionless) characterized by matching symmetry. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances.

Centroid Size/Effect	SS	MS	df	F	<i>p</i>	Pillai tr.	<i>p</i> (Param)
Individual	1,267,588	0.469477	27	12.13	<0.0001		
Side	1,312,120	131.212	1	33.90	<0.0001		
Ind*Side	1,044,953	0.038702	27	1.85	0.026		
Error 1	116,915	0.020888	56				
Shape/Effect							
Individual	0.06646813	0.000175842	378	2.52	<0.0001	9.38	<0.0001
Side	0.00176474	0.000126003	14	1.81	0.0358	0.76	0.0203
Ind*Side	0.0263932	0.000697337	378	3.08	<0.0001	7.57	<0.0001
Error	0.0177762	0.0000226737	784				

Table 2. Procrustes ANOVA for both centroid size and shape from Yamparáez/Sotomayor Population *T. infestans*, (dimensionless) characterized by matching symmetry. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances.

Centroid Size/Effect	SS	MS	df	F	<i>p</i>	Pillai tr.	<i>p</i> (Param)
Individual	46,357,914	1,252,917	37	4.99	<0.0001		
Side	1,439,198	1,439,198	1	5.73	0.0218		
Ind*Side	9,286,172	0.250978	37	20.33	<0.0001		
Error 1	0.938393	0.012347	76				
Shape/Effect							
Individual	0.20439244	0.0003945800	518	2.01	<0.0001	8.81	<0.0001
Side	0.00641765	0.0004584037	14	2.34	0.0039	0.64	0.008
Ind*Side	0.10166526	0.0001962650	518	2.61	<0.0001	9.17	<0.0001
Error	0.08004337	0.000075287	1064				

Table 3. Procrustes ANOVA for both centroid size and shape of Huacaya/Imbochi Population *T. infestans*, (dimensionless) characterized by matching symmetry. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances.

Centroid Size/Effect	SS	MS	df	F	<i>p</i>	Pillai tr.	<i>p</i> (Param)
Individual	661,088,646,217	2,203,628,821	30	1.00	0.4993		
Side	220,987,792	220,987,792	1	1.00	0.3245		
Ind*side	6,606,556,014	2,202,185,338	30	1.00	0.4835		
Error 1	1,363,261,983	219,880,965	62				
Shape/Effect							
Individual	0.10800141	0.000257146	420	4.09	<0.0001	10.75	<0.0001
Side	0.00132375	0.0000945536	14	1.5	0.1065	0.89	<0.0001
Ind*Side	0.02643423	0.0000629386	420	5.28	<0.0001	7.48	<0.0001
Error	0.08004337	0.000075287	1064				

A significant level of FA (Ind*side: $p < 0.0001$) was found in CIPEIN individuals (Table 1), with a regular FA intensity in comparison with the other two populations, suggesting that part of the asymmetry in the F1 (first generation) of the control population (Table 4) is due to genetic influence.

After analyzing the peridomestic populations, FA was found in specimens from Huacaya/Imbochi and Yamparáez/Sotomayor (Ind*side: $p < 0.0001$) (Tables 1 and 2). Nevertheless, after a multivariate regression of Shape vs. Procrustes FA Scores intensity was found to be higher in *T. infestans* from Yamparáez/Sotomayor than specimens from Huacaya/Imbochi (Table 4 and Figure 3).

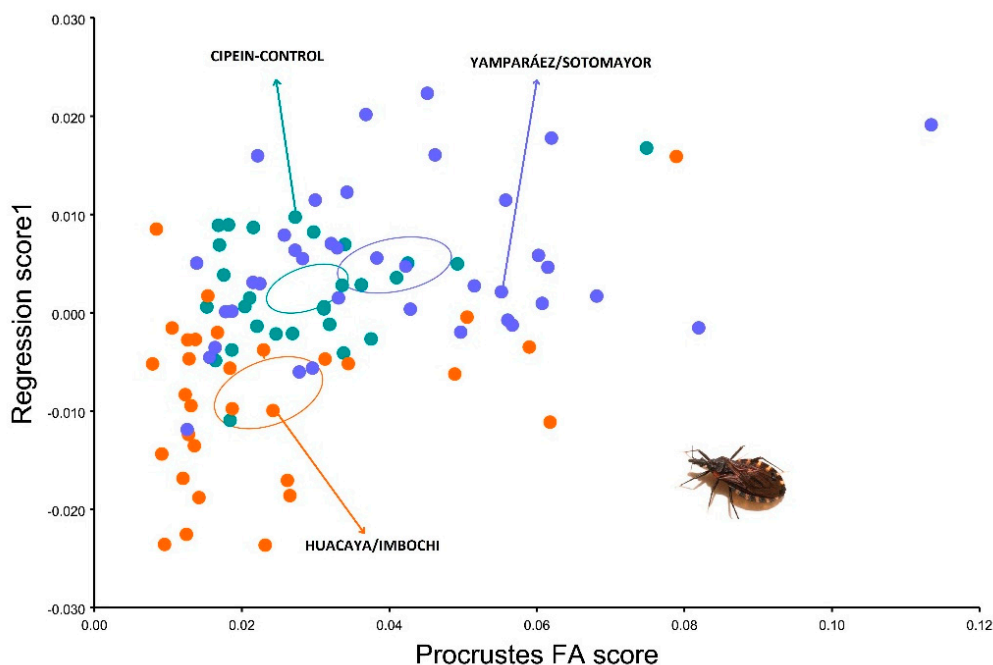


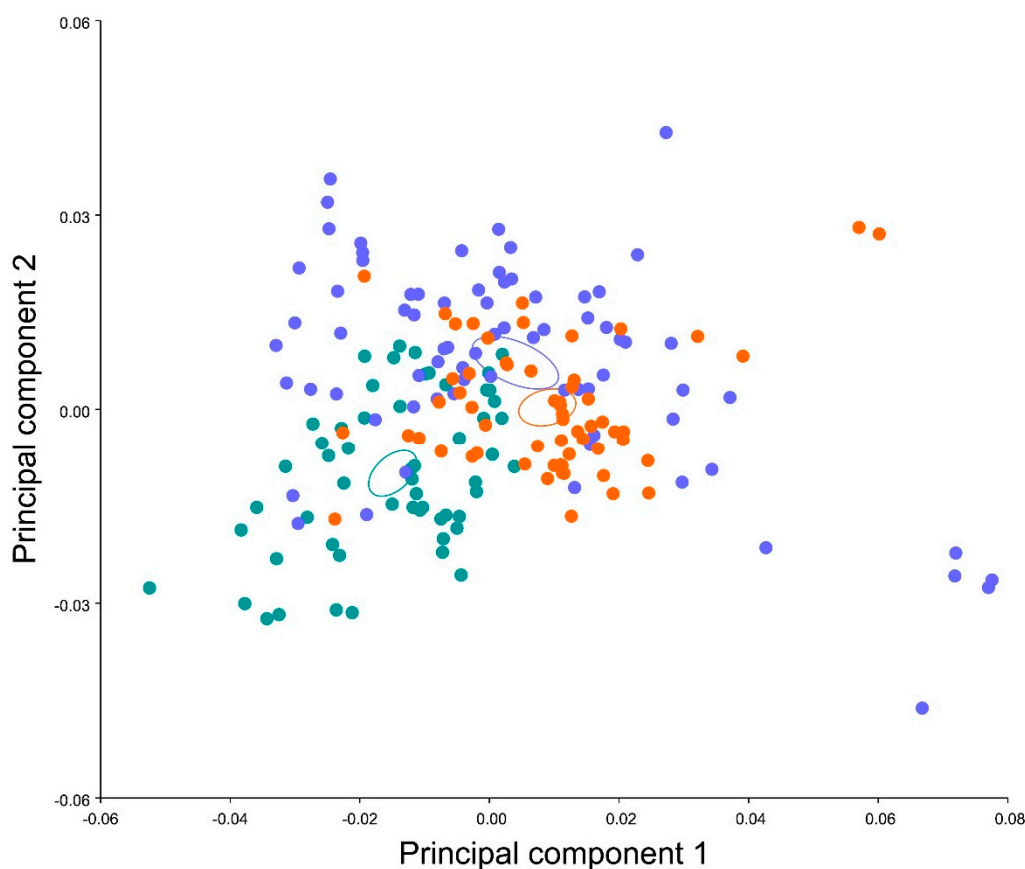
Figure 3. Multivariate regression of Shape as a dependent variable vs. Procrustes FA Scores as an independent variable, showing the intensity of FA between groups. Colors: Green: Cipein-Control, Orange: Huacaya/Imbochi, Blue: Yamparáez/Sotomayor.

Table 4. Intensity of Fluctuating Asymmetry between populations and sex in *T. infestans* Mean Squares (MS) AND Pillai tree data of Procrustes ANOVA of shape.

Population	Sex	N° of Insects	MS (Ind*Side)	Pillai tr.
Yamparáez/Sotomayor	Female	14	0.0001970676	7.98
	Male	24	0.0001892431	8.43
Huacaya/Imbochi	Female	14	0.0000751902	7.05
	Male	17	0.0000546154	6.22
CIPEIN	Female	14	0.0000692699	7.18
	Male	14	0.0000724677	7.04

In order to assess the FA influence by sexes, a Procrustes ANOVA was performed in every population by sex, displaying that females in *T. infestans* from both peridomestic populations showed higher levels of FA compared with male populations (Table 4).

A principal component analysis showed a morphospace where the first three PC's accumulate 56.4% of the shape variation (PC1: 27.8%, PC2: 15.2% PC3: 13.4%), displaying a well-defined shape differentiation between populations. Although Yamparáez/Sotomayor showed more disparity wing shapes in comparison with the other two populations, wing shape varies principally by the movement of the wing veins, displaying wider wings in the populations of Yamparáez/Sotomayor and, on the contrary, more elongated wings with less intraspecific variation in populations of Huacaya/Imbochi were noticeable. Ovalated and had wider wings than the control population of CIPEIN (Figure 4).

**Figure 4.** Principal component analysis of the wing shape between three populations in *T. infestans*. Colors: Green: CIPEIN-Control, Orange: Huacaya/Imbochi, Blue: Yamparáez/Sotomayor.

4. Discussion

It is well known that FA is a measure of developmental instability (DI) [27,51–54]. According to Palmer and Strobeck [29], the visible and measurable asymmetry of right and left sides of the wings of *T. infestans* could be the expression of random disturbances accumulated during development [29]. Populations more exposed to environmental stress show high levels of DI, unlike a “control” population or populations with little exposure [55]. Several stress factors can be present, such as extreme temperatures, food source quality from hosts, stress from environmental pollution and chemical products such as insecticides [21,26,28,33,56–59].

According to Nattero et al. [41], *T. infestans* from open peridomestic habitats are more likely to exhibit FA in the shape compared with domiciliary insects; in addition, FA in insects that inhabit the peridomicile is related to the quality of food sources of hosts, ecotopes and sex. In our study, control population (CIPEIN) were reared under stable laboratory conditions (temperature and humidity) and fed blood from chickens, principally due to the fact that they were not exposed to any insecticide application. This population showed lower values of FA compared with the peridomestic populations from Yamparáez/Sotomayor and Huacaya/Imbochi. Female *T. infestans* from Huacaya/Imbochi have significant differences in FA in wing shape compared with males. According to the characteristics of the goat pens and chicken coops built with sticks and palm trees, and open ecotopes, males have the advantage of flying from the peridomicile to human dwelling buildings because they have predisposition to feed on human blood; however, females are less demanding and limit themselves to staying in pens and chicken coops. On the other hand, the environmental temperature is between 25 to 30 °C in winter, allowing the flight of male triatomines mainly at night [4,6,60].

Similar data were obtained by Nattero et al. (2015) in the location of Figueroa, in the northwest of Argentina. The wing asymmetry patterns of females living in goat pens were significantly different to that of males, because females have less mobility between habitats. A different condition occurs with the *T. infestans* population from Yamparáez/Sotomayor, which presented slightly increased levels of FA in shape and size, compared with the population from Huacaya/Imbochi and the CIPEIN “control” strain. In Yamparáez/Sotomayor the environmental temperature is lower than 15 °C in winter and the humidity is more than 40%, besides the pens and chicken coops are built with blocks of soil, leading to an unfavorable environment for the development of *T. infestans*. These characteristics avoid flying and infesting other peridomestic and domiciliary environments, low temperatures do not allow flight for *T. infestans* [61–63]. Males remain in their habitat, feeding on their hosts like females; this population of female insects showed an increase of FA compared with males. Nattero et al. [42] followed up peridomestic sites in the northwest of Argentina, and showed that FA patterns were not stable in *T. infestans* and depend on the characteristics of habitat and season of the year. Moreover, it seems to be modified by the history of insecticide spraying, either through direct effects on the development of insects or through indirect effects related with flight and invasion of human dwelling habitats.

In our study, the collection of insects was carried out in the peridomicile in winter, six months after insecticide were sprayed in Yamparáez/Sotomayor, and two years after chemical treatment in Huacaya/Imbochi. *T. infestans* from Yamparáez/Sotomayor received chemical treatment with pyrethroid insecticides, (deltamethrin and alpha-cipermethrin) for 17 years (two applications a year) [11], developing resistance according to a research conducted by Lardeux et al. [17] It is possible that regular contacts with insecticides are related with the presence of FA in this population [29]. The FA present in populations collected in natural environments (peridomiciliary) could be related to exposure to the toxic action of insecticides [22,40]. The populations of *T. infestans* from Huacaya/Imbochi were also treated with pyrethroid (deltamethrin) and organophosphates (bendiocarb), but the treatment cycles were less frequent for 17 years (one cycle or less a year) and this insects also developing resistance to pyrethroid insecticides [17]. In Huacaya/Imbochi, chemical control in peridomestic environments has been historically difficult because of cultural

practices and the low availability of chemicals [11]. Furthermore, the construction of pens and chicken coops is precarious and the insecticide is easily eliminated by environmental factors, such as rain and solar rays, which contribute to the degradation of insecticides [64]. Moreover, in phenological stages such as eggs, the insecticide is not effective, and the insects survive and reinfest [65]. However, insecticides have a repellent effect and *T. infestans* inhabit other peridomestic environments [60]. A study conducted by Nattero et al. [40] indicates that before fumigations, FA levels in wings of *T. infestans* were higher than after chemical treatment, mentioning that this pattern may be related to a selective survival adaptation to insecticides, that may or may not be mediated by resistance to pyrethroids and may be associated with feeding success. Genetic disturbances include intense directional selection and certain specific genes [66]. Both populations have been in constant contact with insecticides, developing a resistance to pyrethroids. Lardeux et al. [17] carried out a biological test to detect the sensitivity to deltamethrin of the populations of *T. infestans* from Yamparáez/Sotomayor and Huacaya, reporting 58% and 67%, respectively.

5. Conclusions

The following research confirms the presence of developmental instability in *T. infestans* by the quantification of FA. *T. infestans* was found to be sensible for the environmental conditions from the different peridomestic population studied, and was also found to be sensible for insecticide application. The results were found to relate the levels of FA to places more exposed to insecticides during a sustained period of time. There are still no reports of the identification of genes for resistance to insecticides in both populations. Although FA was detected in both populations studied, the population of *T. infestans* from Yamparáez/Sotomayor presented higher levels of FA that could be associated with higher levels of stress related to environmental characteristic, microhabitat and greater exposure to insecticides. Therefore, the combination of environmental characteristics such as temperature, relative humidity of the environment, preference in feeding according to the source, characteristics of peridomestic habitats, and resistance to insecticides, are one of the main factors that influence the development of FA in wings in populations of *T. infestans* from Yamparáez/Sotomayor and Huacaya/Imbochi, regions in Chuquisaca Bolivia. More multifactorial studies are needed with the combination of genomic and transcriptomic analyses which can relate the particular substance of the insecticide to the levels of FA in the different populations, which are the next steps for this research.

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