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Research Article

Morphometric Analysis of the Host Effect on Phenotypical Variation of *Belminus ferroae* (Hemiptera: Triatominae)

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The Triatominae subfamily includes hematophagous insects, well known for their role as vectors for the *Trypanosoma cruzi* parasite, etiologic agent of Chagas' disease. *Belminus ferroae* is a triatomine that showed an increased demographic fitness when cockroaches were used as hosts. Here we compare the centroid size (CS) and wing shape between *B. ferroae* parents and three successive generations (O1, O2, and O3) of their offspring fed on cockroaches or mice under laboratory conditions. Morphometric analysis of the wings bugs fed on cockroaches showed a significant reduction in CS in both sexes among all generations. Sexual size dimorphism (SSD) was observed in the insects fed on cockroaches (O2 and O3), as well as those bugs fed on mice (O2). Differences in the shape of wings were observed between parental and offspring wings when fed on mice, but not in males (O1, O2, and O3) or females (O1 and O2) fed on cockroaches. There was a greater wing shape similarity between the cockroach-fed offspring and their parents according to the Mahalanobis distances. Our results support the idea of higher adaptation of this Triatominae with arthropod hosts.

1. Introduction

The Triatominae are true bugs comprising a subfamily of Reduviidae (Hemiptera: Heteroptera) with 147 species [1]. The members of this subfamily are almost entirely hematophagous, although some species feed on other invertebrates [2, 3]. The genus *Belminus* belongs to this taxonomic group comprised of eight species found in Mexico, Costa Rica, Panama, Colombia, Venezuela, Peru, and Brazil [4]. Their feeding behavior includes cannibalism, cleptohematophagy, hemolymphagy, and hematophagy [5–8].

It has been suggested that arthropods could be principal food sources of *Belminus ferroae* and cockroaches have been implicated as probable hosts within human dwellings [4]. Recently comparison of cohorts of this species fed on arthropods (*Blaberus* and *R. prolixus*) and vertebrates (mice) showed for the first time the existence of a triatomine not restricted to bloodsucking. Moreover, feeding on cockroaches offered clear demographic advantages, namely, higher intrinsic rate of natural increase, finite rate of population growth, and net reproductive rate [2].

Our current study analyzes *Belminus* form by measuring variations of wing shape and centroid size between *B. ferroae* individuals captured in human dwellings and three successive offspring generations reared under laboratory conditions. By associating changes in these variables (i.e., wing centroid size and shape) with the different hosts, we evaluate the premise that adaptation to a food source produces greater morphologic similarity (of size and shape) between parents and their offspring. Although this is our principal hypothesis, alternative explanations are also discussed to account for unexpected results. Among other analytic tools used, geometric morphometry was chosen for the usefulness it was shown to have in identifying the habitat origin of reinfesting triatomines [9] and in detecting the effects of hosts in a variety of insect groups [10, 11].

2. Materials and Methods

2.1. Insects. The parental insects used in this study were captured in dwellings located in Toledo municipality of the Department of Norte de Santander, Colombia. The region is characterized by a humid premontane forest landscape, with a range of altitudes between 1190 and 1499 m.a.s.l., an average annual temperature of 25°C, and a relative humidity of 85% [4].

2.2. Maintenance of the Parental Insects. A total of 35 individuals (14 males and 21 females), captured in the field, were organized in eight containers, putting one or two males and two or three females in each. These containers were divided into two groups, four belonging to the group fed on mice and four belonging to the group fed on cockroaches. In the mice-fed group, the insects were fed on sedated (Ketalar 75 mg/kg) hosts for 3 h once a week. The cockroach-fed group was maintained with adults of *Blaberus giganteus.* The colony of cockroaches was founded from specimens collected from the same area as the *B. ferroae*. They were replaced monthly and maintained with a mixture of rabbit food, corn, and moist cotton. Plastic containers of 10 × 7 × 2 cm with absorbent paper inside were used to raise the insects; the lids covering the containers were perforated to prevent water condensation and mold.

2.3. Maintenance of Offspring. Containers with parental insects were checked weekly to extract and transfer the eggs to new breeding recipients at a density of 10 insects per container, until adults emerged as offspring 1 (O1). Then, random couples were organized, one to each of 30 containers. Fifteen couples were fed on mice and fifteen on cockroaches, and their eggs gave rise to offspring 2 (O2), and this process continued until offspring 3 (O3). The feeding scheme for offspring fed on mice was similar to that used for the parents. For offspring fed on cockroaches, the first, second, and third instar nymphs of *B. ferroae* were kept with nymphs of *B. giganteus* (fourth or fifth instar), and the elder instars were kept with an adult cockroach.

The experimental breeding was carried out in an acclimatized cabin with constant temperature (25 ± 2°C), relative humidity (70 ± 10% RH), and photoperiod (12:12 h light/dark). The procedures of animal management were carried out in accordance with the Colombian law (National Animal Protection Statute, Law 84, 1989, and Resolution 8430, 1993, of the Colombian Health Ministry).

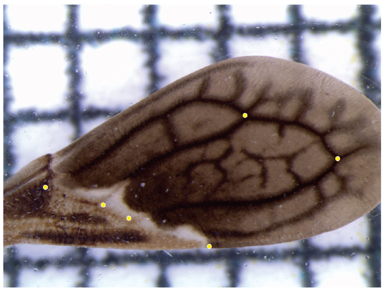


FIGURE 1: Six landmark positions on the left wing of *B. ferroae* used in this study.

2.4. Wing Mounting. Both fore wings of each specimen were removed and mounted in a dorsal position on a slide under a stereoscopic microscope (M205 C, Leica). The wings were mounted between a microscope slide and a cover slip adhered by its edges with liquid silicone. Each specimen was assigned a code which was registered for each slide. Relative data (i.e., date of mounting, number of wings, sex, and code of breeding container) were saved on a spreadsheet.

2.5. Image Capture and Digitalization. A total of 590 left wings were photographed with a CMOS camera (EC3, Leica) coupled to the stereoscope, each one three times. Six landmarks were chosen and all belonged to *type 1* [12] (see Figure 1). The photographs were exported and landmarks were digitized with the software tpsDig (version 2.7) to obtain the geometric coordinates. Preparation of the input files to tpsDig as well as for file organization and conversions afterwards was performed using the software tpsUtil (version 1.47).

2.6. Error Estimates. To reduce the error in the digitalization of the landmarks, this work was done by a single person. The error was estimated by means of the index of repeatability, as described by Arnqvist and Mårtensson [13], which uses a one-way analysis of variance (ANOVA) with repeated measurements, where "*R*" indicates the quotient between the individual variation and the total variance [14]. Parents and their corresponding O1 reared on cockroaches or mice were included in this analysis. The test was carried out with VAR software on each sex separately.

2.7. Size Variation. For comparison of the overall wing size between parents and offspring, the isometric estimator known as "centroid size" (CS) was used, which is defined as the square root of the sum of the squared distances between the centroid of the configuration of landmarks and each individual landmark [15]. The CS was calculated using a package program for doing geometric morphometrics (MorphoJ 1.05e software) [16]. Sexual size dimorphism (SSD) and metric changes in CS were analyzed for each sex using the Kruskal-Wallis test, and the statistical significance was adjusted according to the Bonferroni correction (PAST 3.01 software).

2.8. Shape Variation. The shape variables (Procrustes coordinates) were obtained using the MorphoJ program, which uses the generalized Procrustes analysis (GPA) that is a superimposing algorithm [16]. Nonetheless, since the number of variables exceeds the number of degrees of freedom, the Procrustes coordinates cannot be used as standard variables in multivariate statistical tests. For this reason a nonparametric alternative involving resampling procedures as bootstrap and permutation was necessary [12, 17]. These analyses do not demand estimation of the degrees of freedom.

In order to compare the variations of shape between the parents captured in the field and the offspring reared in the laboratory with the different hosts, a nonparametric MANOVA (NPMANOVA) was carried out using statistical software (PAST 3.01). Then, canonical variate analysis (CVA), which computes the Mahalanobis distances based on all the groups incorporated in the study, was performed (MorphoJ software). Additionally, a discriminant analysis (DA) was executed to compare each pair of groups separately and to calculate percentages of reclassification and cross-checking. All tests were carried out with 10,000 permutations and the statistical significance was adjusted according to the Bonferroni correction [12, 16].

2.9. Allometry. The relationship between CS and shape variation was examined by multivariate regression (MorphoJ software). The statistical significance of this test was estimated by permutations using 10,000 runs [16].

2.10. Size Correction. The procedure proposed by Viscosi and Cardini [18] was used to verify if wing shape differences were due only to variations in size, or if the variability of shape was affected by the feeding treatments. This method consisted in examining the differences in the allometric pattern (i.e., the slopes) on all offsprings by sex. These differences were analyzed using a full multivariate covariance analysis (MANCOVA) model, with "groups" as the predictor factor and "CS" as the covariate (SPSS 19 software). The interaction term $group \times CS$ was used to evaluate the differences between the slopes of the allometric trajectories between different groups of offsprings [12]. The statistical significance was estimated using 1,000 bootstraps (SPSS 19 software).

In those groups, where it was clear that no significant differences existed in the allometric patterns, the correction for size was carried out by means of a pooled regression within subgroups O1, O2, and O3; shape was used as dependent variable and CS as covariate (MorphoJ). This allowed removal of the within-offspring allometric variation and computation of the regression residuals subjected to other "after-correction" statistical analyses (i.e., NPMANOVA, CVA, and DA).

2.11. Visualizing Shape Variation. An analysis of shape variation was performed using a computer equipped with software (MorphoJ). A wireframe is a set of lines (wires) connecting the digitized points (landmarks) on a shape. The soft wireframe graphs show shifts of the landmarks from the starting to the target shape [19]. The first wireframe was drawn on the starting shape (in our case, the shape parent is shown in light blue; see Figures 4 and 5). A second wireframe is drawn on the target shape, which in this study corresponds to the offspring shape (O1, O2, and O3) and permits seeing the result of the deformation caused on the starting shape (dark blue, see Figures 4 and 5). The vertices of the wireframes show where the landmarks of the two shapes differ in location. If the wires in the target shape appear as curves, this reflects the deformation of the space between landmarks as inferred from the techniques employed with the thin-plate spline [20]. To better represent the shape change, the scale factor was increased fivefold [18].

2.12. Program Sources. tpsDig2 and tpsUtil are to be found in http://life.bio.sunysb.edu/morph/, MorphoJ in http://www.flywings.org.uk/morphoj_page.htm, PAST in http://folk.uio.no/ohammer/past/, and VAR in http://mome-clic.com/clic-collection/clicmodules/var.

3. Results

3.1. Error Estimation. Comparison of three repeated sets of photographs of the same set of wings showed fairly good agreement for the centroid size on females ($n = 94$, $R = 0.992$, 0.993, and 0.992) and males ($n = 99$, $R = 0.992$, 0.990, and 0.991).

3.2. Size Variation. The size variation study was performed on a total of 590 adult insects (305 females and 285 males). All analyses were carried out separately for each sex. The numbers of wings (n), mean, and standard deviation of CS for each group classified by host and by sex are detailed in Table 1.

The CS of parental wings of either sex was larger than those of their offspring (Table 1), although metric differences were statistically significant only for offspring fed on cockroaches (Table 2). Differences were statistically significant between contemporaneous offspring feeding on different hosts, in females and males from O2 to O3 (Table 2). Wing CS differences between noncontemporaneous offspring fed on the same host were not found, except between males (O1-O2) fed on mice (Table 2).

Kruskal-Wallis comparison between females and males did not show statistically significant differences in SSD among parents ($p = 0.63$), O1 fed on cockroaches ($p = 0.75$), and O1 ($p = 0.0076$) and O3 ($p = 0.036$) fed on mice. However, significant SSD was observed in the O2 ($p = 0.000044$) and O3 ($p = 0.0009$) fed on cockroaches and in the O2 ($p = 0.00046$) fed on mice (Figure 2).

3.3. Allometry. The relationship between wing shape variables and CS showed a significant but low allometric residue: 7.4% in females ($p < 0.001$) and 2.9% in males ($p = 0.0006$).

3.4. Shape Variation. The NPMANOVA of the shape variables (Procrustes coordinates) between parental and female ($F = 19.01$; $p = 0.0001$) or male ($F = 16.55$; $p = 0.0001$) offspring rejected the null hypothesis that the means do not differ. The canonical analysis of the variance in females demonstrated that the first canonical function explained 88% of the total variance and the second function 8%

Table 1: The numbers of specimens (n), mean, and standard deviation of centroid size for each group classified by host and sex.

Group	Origin	Sex	Feeding behavior	O	n	Centroid size			
						Mean	Min	Max	SD
P	Field	♀		P	18	1455.84	1361.90	1607.30	67.04
HLO1	Laboratory	♀	Hemolymphagy	O1	45	1341.65	1219.31	1461.48	56.20
HLO2	Laboratory	♀	Hemolymphagy	O2	67	1356.23	1132.53	1481.38	62.30
HLO3	Laboratory	♀	Hemolymphagy	O3	69	1330.59	1230.69	1474.11	45.43
HMO1	Laboratory	♀	Hematophagy	O1	29	1394.37	1297.15	1496.69	52.31
HMO2	Laboratory	♀	Hematophagy	O2	57	1417.21	1228.20	1510.00	57.24
HMO3	Laboratory	♀	Hematophagy	O3	20	1404.68	1238.31	1493.80	58.00
P	Field	♂		P	14	1439.57	1360.48	1512.14	42.59
HLO1	Laboratory	♂	Hemolymphagy	O1	38	1337.52	1154.50	1467.12	62.59
HLO2	Laboratory	♂	Hemolymphagy	O2	71	1314.32	1191.50	1433.39	53.94
HLO3	Laboratory	♂	Hemolymphagy	O3	65	1299.48	1119.23	1403.64	55.65
HMO1	Laboratory	♂	Hematophagy	O1	44	1361.18	1223.34	1476.33	49.83
HMO2	Laboratory	♂	Hematophagy	O2	36	1378.53	1250.72	1461.85	46.44
HMO3	Laboratory	♂	Hematophagy	O3	17	1367.64	1249.47	1469.83	60.27

P (parental), O (offspring), ♀ (female), ♂ (male), O1 (first offspring), O2 (second Offspring), O3 (third offspring), HL (hemolymphagy = fed on cockroaches), HM (hematophagy = fed on mice), n (number of specimens), Min (minimum), Max (maximum), and SD (standard deviation).

Table 2: Comparison of centroid size of *B. ferroae,* between parental and progeny reared under laboratory conditions with two different hosts: cockroaches and mice (Kruskal-Wallis test).

Comparison	Sex	
	Females	Males
Parental versus offspring		
P-HLO1	$7.5E - 07^{*}$	$1.81E - 04^{*}$
P-HLO2	$1.30E - 05^{*}$	$8.17E - 09^{*}$
P-HLO3	$3.40E - 10^{*}$	$9.7E - 11^{*}$
P-HMO1	0.3149	0.0201
P-HMO2	1.0000	0.3868
P-HMO3	1.0000	0.1153
Contemporaneous offspring (reared on different host)		
HLO1-HMO1	0.01481	1.000000
HLO2-HMO2	$4.7E - 06^{*}$	$2.22E - 06^{*}$
HLO3-HMO3	0.00002^{*}	0.00301^{*}
Offspring noncontemporaneous (reared on the same host)		
HLO1-HLO2	1.000000	0.607057
HLO1-HLO3	1.000000	0.032183
HLO2-HLO3	0.154945	1.000000
HMO1-HMO2	1.000000	0.000435^{*}
HMO1-HMO3	1.000000	1.000000
HMO2-HMO3	1.000000	1.000000

P (parental), HL (hemolymphagy = fed on cockroaches), HM (hematophagy = fed on mice), O1 (first offspring), O2 (second offspring), O3 (third offspring), and *statistically significant difference, $p < 0.0033$.

(Figure 3(a)). In males the first canonical function explained 84% and the second 8% (Figure 3(b)). The Mahalanobis distances between wing shapes of male offspring fed on cockroaches and their parents did not show significant statistical differences. However, female offspring did do so from O2 to O3. All the distances between the wing shapes of all offspring fed on mice and their respective parents were statistically significant (Table 3).

The Mahalanobis distances in wing shape were statistically significant between all offspring feeding on different hosts (Table 3), while differences in wing shape between offspring fed on the same host did not occur (Table 3).

In female offspring, the MANCOVA showed significant differences between slopes (Pillai's trace 0.239; $F = 1.717$; df1 40, df2 1.360; $p = 0.004$), while in males no such differences were observed (Pillai's trace 0.184; $F = 1.221$; df1 40, df2

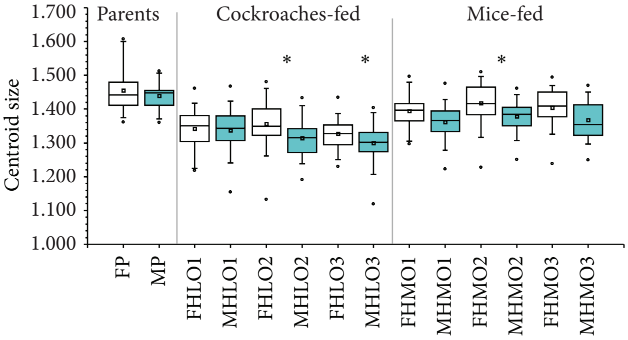


Figure 2: Sexual dimorphism in centroid size. FP (♀ parent), MP (♂ parent), FHLO1 (♀O1, hemolymphagy), MHLO1 (♂O1, hemolymphagy), FHMO1 (♀O1, hematophagy), MHMO1 (♂O1, hematophagy), FHLO2 (♀O2, hemolymphagy), MHLO2 (♂O2, hemolymphagy), FHMO2 (♀O2, hematophagy), MHMO2 (♂O2, hematophagy), FHLO3 (♀O3, hemolymphagy), MHLO3 (♂O3, hemolymphagy), FHMO3 (♀O3, hematophagy), MHMO3 (♂O3, hematophagy), O1 (first offspring), O2 (second offspring), and O3 (third offspring). White box (female) and blue box (male). *Statistically significant difference.

1.280; $p = 0.164$). This result showed that size correction could be calculated only for males. The NPMANOVA of the size-corrected shape variables (residuals) between male offspring ($F = 4.52$; $p = 0.0001$) rejects the null hypothesis that the means do not differ between groups. When these residuals were subjected to the CVA, the first canonical function explained 89% of the total variance and the second function explained 5% (Figure 3(c)). All the Mahalanobis distances between male offspring fed on different hosts were statistically significant (Table 3).

3.5. Visualizing Shape Variation. Figures 4 and 5 illustrate the relative changes in wing shape of males and females. The reference or starting configuration in this particular case (sky blue) describes the parental configuration, while the dark blue shows the deformation produced in the parental configuration by superimposing on it the configuration of the offspring. The compared pairs refer to the same ones used in the DA (Table 3). This graph highlights that the differences between the parental configurations and those generations fed on cockroaches are slight. Shape changes' tendencies were similar in females and males of each group raised with a certain host. The changes were located mainly in landmarks 2 and 4 when comparing parents and offspring fed on cockroaches, whereas in the case of insects fed on mice, the relative displacements were located in landmarks 1, 2, 3, and 4 (Figures 4 and 5).

Deformations to the parental configuration of female offspring fed on cockroaches were more pronounced than in males fed on the same host (Figures 4 and 5). The opposite occurred in the insects fed on mice. This graphic result can be verified by means of percentages of reclassification and crosschecking provided by the DA (Table 3).

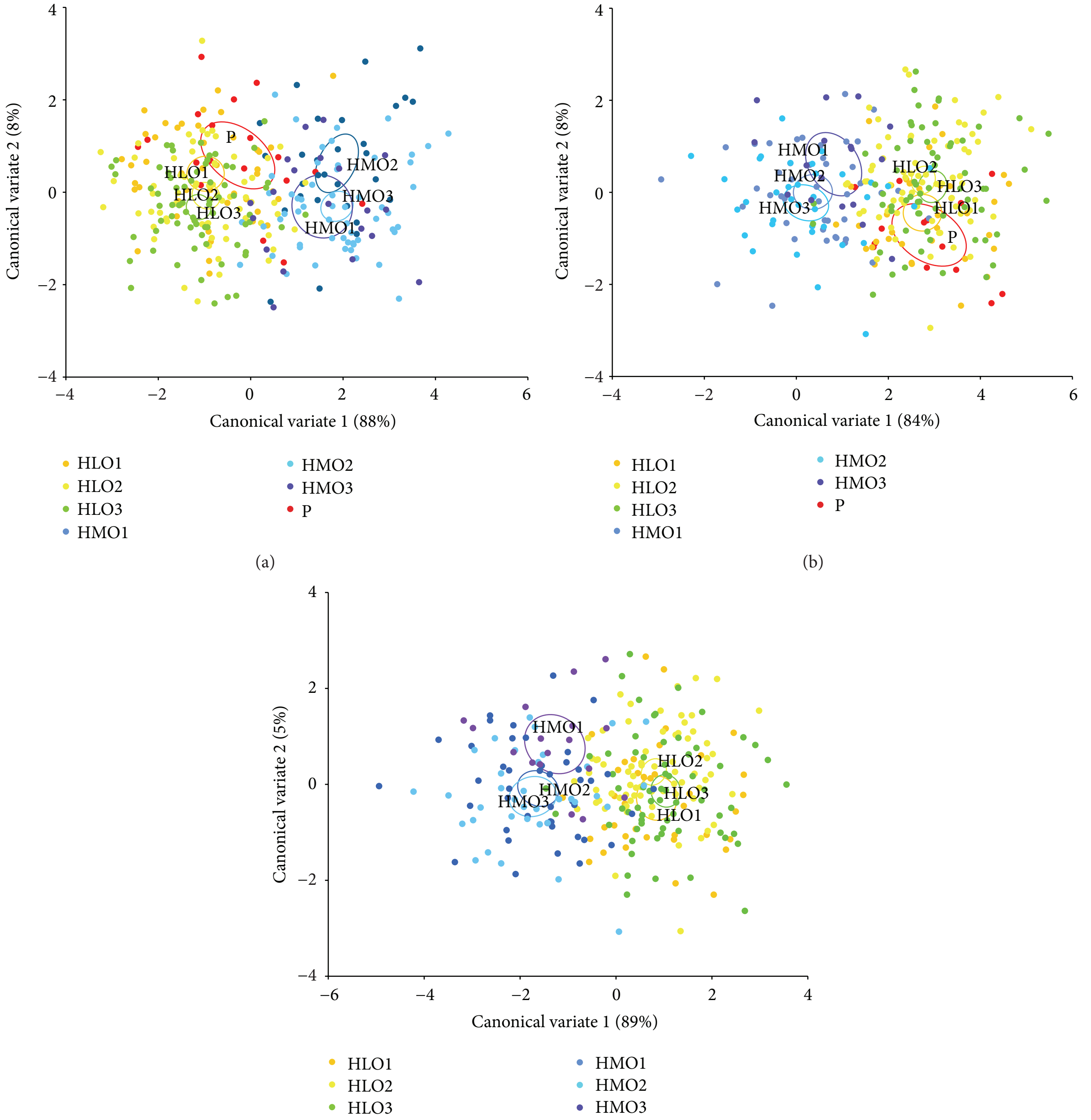
4. Discussion

4.1. Size Variation. There was an obvious tendency towards wing reduction in both males and females of *B. ferroae* raised with either host (cockroaches or rodents). Nonetheless such reduction was only significant in those insects fed on cockroaches (Figure 2, Table 2).

For adaptations reasons, it would be reasonable to expect greater morphological stability through similarity in size in individuals fed on cockroaches. However, since *B. ferroae* fed on cockroaches reduced wing size from parents to their offspring, an alternative explanation must be considered.

It is common to find that among *Rhodnius*, *Triatoma*, and *Panstrongylus* genera body size decreases when individuals are laboratory reared for several generations or their habitat changes from sylvan to domestic [14, 21–24]. As far as diet is concerned, wing size variation under natural conditions has been related to the nutritional value of vertebrate hosts [25, 26]. Based on the analysis of the contents of their intestines, *B. ferroae* captured in houses seem to prefer cockroaches as their principal host [4]. This finding is consistent with some demographic analyses where fitness was assessed [2]. But the possibility of a different nutritional value of the invertebrate hosts of *B. ferroae* found in the field and those offered in the laboratory could help to explain the contrasts found in size between parents and offspring fed on cockroaches. In this study, *B. ferroae* was collected together with blatids in rural domiciles where *Periplaneta americana* were present. However, in our study another cooccurring cockroach species (genus *Blaberus*) was used [4].

The experimental design used in this study included differences in host-feeding frequency, daily for the insects fed cockroaches and weekly for those fed on mice. Therefore, heterogeneity in host availability might explain the differences in size, supposing a higher consumption of food in the insects reared on cockroaches. However, one would suppose that bugs fed on mice ingested more food, based on better performance of some component of fecundity such as rhythm (total eggs/female/week during the reproductive period) and intermittence of oviposture (average time between two successive oviposture), in contrast to insects fed on cockroaches, that is, although the last have greater access to the host [2]. For these reasons, one might consider that other dietary factors like host preference must affect growth of the bugs.



(a)

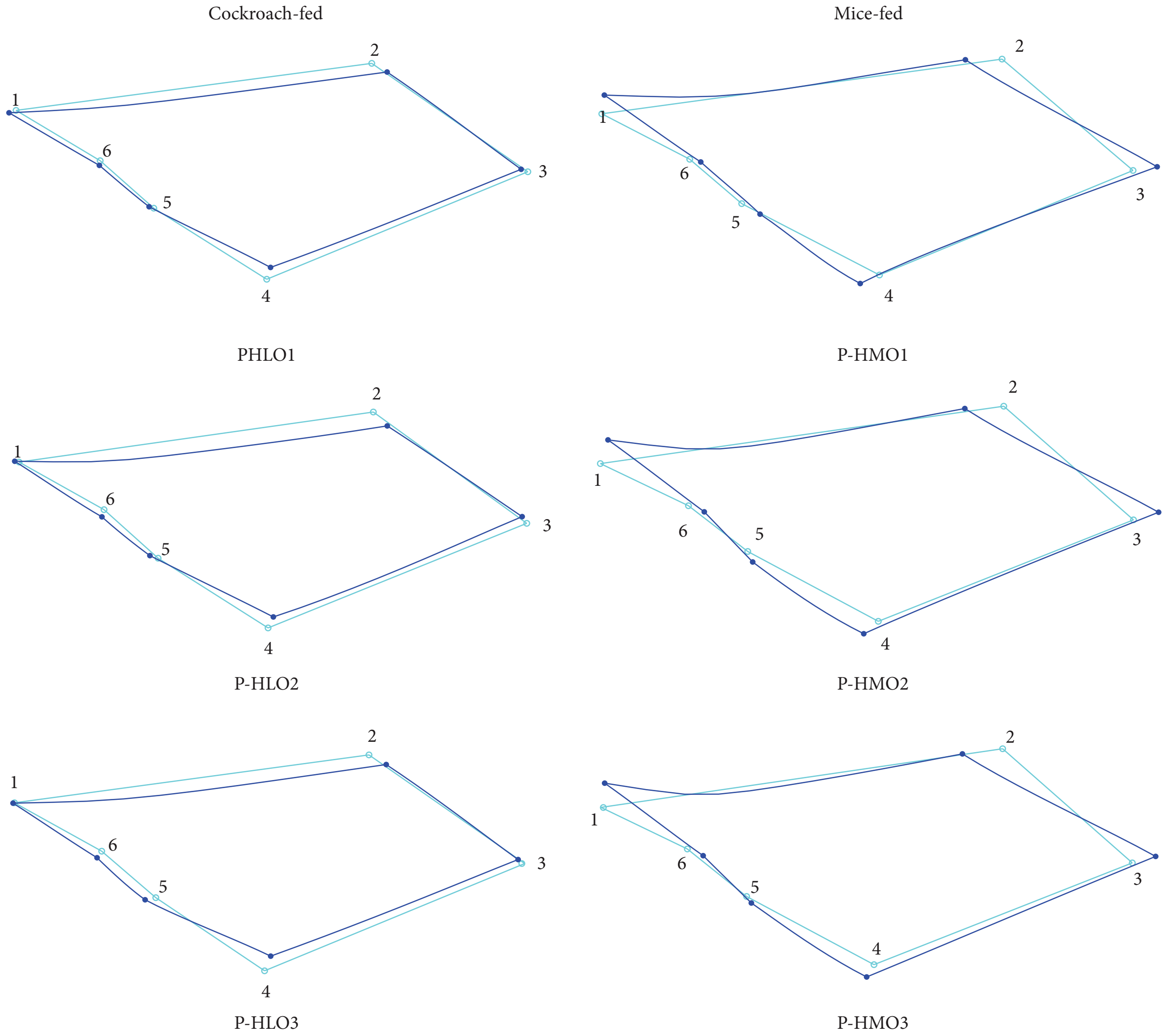
(b)

(c)

FIGURE 3: Factorial maps in the plane of the two canonical factors of shape variation for wing (CV1 and CV2) presenting the distribution of the phenotypes of wing shape in females (a), males (b), and (c) males offspring (after size correction). The ellipses represent the confidence interval of the average shape of each group with a probability of 95%. P (parent), HL (hemolymphagy = fed on cockroaches), HM (hematophagy = fed on mice), O1 (first offspring), O2 (second offspring), and O3 (third offspring).

Guarneri et al. [27] suggest that the life cycles of *T. infestans* and *T. brasiliensis* bugs were shorter in those fed on mice than in those fed on bird, probably due to their natural history with the mammal. In our study some key aspects related to blood diet adaptation, such as antihemostatic factors, digestive enzyme efficiency [28], evolution of mechanisms to confront the effects of oxidative stress [29], and the adequate contribution of nutritional factors like vitamin B [30], could be involved in the delayed development time of bugs fed on mice and consequently in the frequency of larger sized individuals in this group.

Size differences among the contemporaneous offspring of *B. ferroae* fed on cockroaches or mice might be explained by the "growth hypothesis" [21], which proposes that long life


Cockroach-fed

Mice-fed

PHLO1

P-HMO1

P-HLO2

P-HMO2

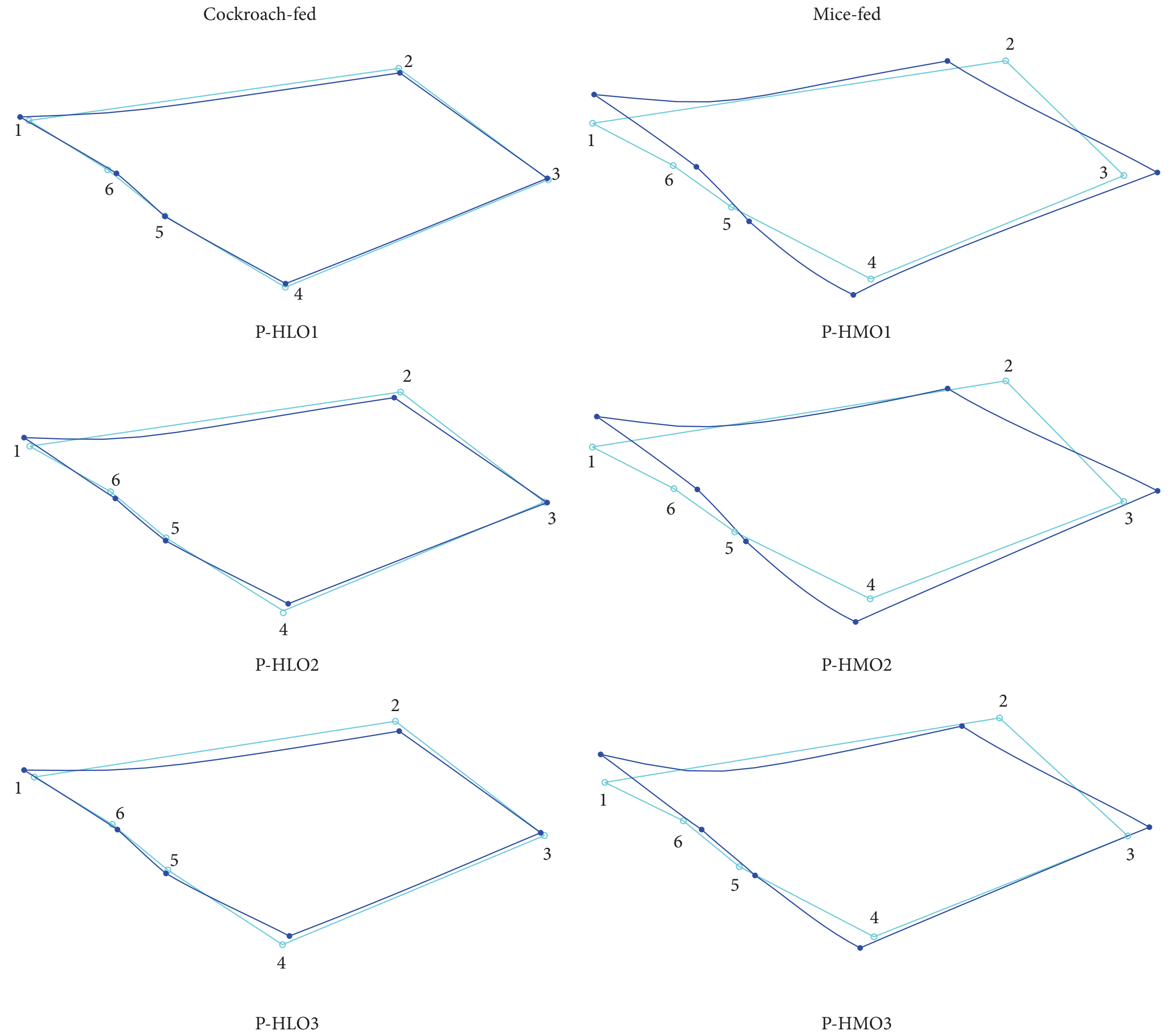
P-HLO3

P-HMO3

Figure 4: Wing deformation caused by superimposing the offspring shape on the parental shape. The sky blue configuration refers to the average shape of the parental females and the dark blue one to the deformation of the parental shape introduced by the laboratory-bred females. Points 1 through 6 refer to the landmarks used to define the wing configuration. (P) Parental, HL (hemolymphagy = fed on cockroaches), HM (hematophagy = fed on mice), O1 (first offspring), O2 (second offspring), and O3 (third offspring).

spans generate large insects and short spans result in small insects. In accordance with this, Nijhout [31] points out that the control of body size is not so much a control of growth but a control of when to stop growing. Final body size is fixed principally by the time available for growth [25, 32]. Some results of the life cycle of *B. ferroae* support this hypothesis, as development time from nymph to adult was significantly shorter ($p < 0.001$) in the insects fed on cockroaches (135.2–144.2 days) than in those fed on mice (187.0–203.5 days) [2].

Finally, it is important to point out that our experimental conditions were not the same as those that the insects find upon infesting human dwellings: availability of hosts, diversity of hosts offered (arthropods and vertebrates), and other factors, for example, photoperiod, temperature, and relative humidity, all likely influenced the size of the insects [23, 25]. In general, the principal factors that affect body size in insects under laboratory conditions are temperature, crowding, endogamy, diet (nutrient quality), and host [10, 14, 25, 33–35]. As temperature and crowding were constant in our current study, size differences among contemporaneous offspring fed on cockroaches or mice cannot be ascribed to variations in these factors. Similarly, the endogamy does not seem to have an important influence because no significant size differences were found between noncontemporaneous offspring reared with either host. Our results showed that size was stabilized from the first generation on similar to observations found in *P. geniculatus* [23].


Cockroach-fed

Mice-fed

P-HLO1

P-HMO1

P-HLO2

P-HMO2

P-HLO3

P-HMO3

Figure 5: Wing deformation caused by superimposing the offspring shape on the parental shape. The sky blue configuration refers to the average shape of the parental males and the dark blue one to the deformation of the parental shape introduced by the laboratory-bred males. Points 1 through 6 refer to the landmark used to define the wing configuration. (P) Parental, HL (hemolymphagy = fed on cockroaches), HM (hematophagy = fed on mice), O1 (first offspring), O2 (second offspring), and O3 (third offspring).

Sexual size dimorphism (SSD) in Triatominae is well known as females are generally larger than males [36]. Nijhout [31] points out that large individuals store larger and better resources than the smaller ones. A large body could be more useful to a female than to a male because it increases the probability of survival and production of a greater number of eggs [37, 38]. SSD is frequently considered adaptive in reproduction, as larger individuals would have a greater opportunity to be chosen by their mate [38].

However, within a species, the degree of SSD may be affected by environmental conditions [39]. Quantitative analysis of changes in size of species like *R. domesticus*, *R. prolixus*, and *T. infestans* suggests that reduction in the average size of females may lead to diminished sexual dimorphism [22] as a result of more stable conditions found in human dwellings or laboratories. Thus, reduction in this morphological character has been proposed as a marker of domiciliation in Triatominae [22–24]. The fact that SSD persists in *P. geniculatus* after five laboratory generations suggests that more generations are necessary for this phenomenon to be manifested [23].

The absence of SSD in *B. ferroae* collected in human dwellings could be interpreted as evidence that adaptation to this environment is not recent and that more than five generations had been raised there. However, the presence in this study of SSD in offspring of *B. ferroae* fed on either cockroaches or mice shows that under stable breeding conditions SSD certainly may occur. Therefore, in spite of the consistent size reduction of the offspring of *B. ferroae*, it is

TABLE 3: Comparison of wing shape of *B. ferroae,* between parental and offspring reared under laboratory conditions with two hosts (cockroaches and mice). Mahalanobis distances (Md) and their significance (p) obtained from CVA and classifications and cross-check from DA.

	Females				Males							
Comparison	Procrustes coordinates								Residuals (after size correction)			
	Md	p	% Classif.	% Cross-check	Md	p	% Classif.	% Cross-check	Md	p	% Classif.	% Cross-check
Parental versus offspring												
P-HLO1	1.0127	0.175	77–66	50–60	0.8561	0.4955	71–63	43–58				
P-HLO2	1.3408	0.003	72–81	55–76	1.2671	0.0103	71–73	50–70				
P-HLO3	1.5354	<0.0001*	72–80	66–78	1.3038	0.0389	79–74	50–68				
P-HMO1	2.338	<0.0001*	89–96	78–86	2.7723	<0.0001*	100–93	79–90				
P-HMO2	2.4613	<0.0001*	78–91	72–82	2.8637	<0.0001*	86–92	64–81				
P-HMO3	2.2396	<0.0001*	83–95	66–70	2.658	<0.0001*	79–100	64–88				
Offspring (reared on different host)												
HLO1-HMO1	2.8991	<0.0001*	93–100	82–97	2.4688	<0.0001*	90–90	87–83	2.5807	<0.0001*	55–64	55–64
HLO1-HMO2	2.9373	<0.0001*	91–91	87–89	2.6063	<0.0001*	92–92	84-83	2.7678	<0.0001*	66-67	66-67
HLO1-HMO3	2.662	<0.0001*	89–95	84–90	2.2699	<0.0001*	90–94	87-88	2.4968	<0.0001*	55–53	55–53
HLO2-HMO1	2.9739	<0.0001*	96–89	91–86	2.4497	<0.0001*	92-91	87–90	2.612	<0.0001*	70–73	69–73
HLO2-HMO2	2.8772	<0.0001*	90–88	89–86	2.6409	<0.0001*	93–89	92–86	2.8634	<0.0001*	77–75	77–75
HLO2-HMO3	2.5492	<0.0001*	89-90	85–80	2.1027	<0.0001*	89-88	85–77	2.4354	<0.0001*	73–65	71–65
HLO3-HMO1	3.1613	<0.0001*	94–97	93–86	2.6471	<0.0001*	94–89	94–84	2.9286	<0.0001*	74–80	74–80
HLO3-HMO2	3.0115	<0.0001*	94-93	94–84	2.7983	<0.0001*	92–89	91–89	3.1581	<0.0001*	83–81	83–81
HLO3-HMO3	2.7788	<0.0001*	97–100	96–90	2.3596	<0.0001*	94–89	94–100	2.8389	<0.0001*	80–65	80–65
Offspring (reared on the same host)												
HLO1-HLO2	0.6264	0.2769	62–64	51–58	0.8409	0.0122	58–70	56–62	0.866	0.0151	66–56	66–55
HLO1-HLO3	0.8455	0.0095	60–64	53–58	0.7487	0.1196	58–63	47–54	0.9359	0.018	66–60	66–60
HLO2-HLO3	0.7086	0.015	69–67	66-65	0.5363	0.2792	61–64	55–52	0.6454	0.1183	55–52	55–51
HMO1-HMO2	1.0607	0.0099	72–72	58–64	0.7865	0.1514	75–64	59–50	0.8317	0.1515	52–58	52–58
HMO1-HMO3	1.2678	0.0416	69–80	62–60	1.1701	0.0289	80–71	80–53	1.2126	0.0403	50–41	50–35
HMO2-HMO3	0.6175	0.6581	71–60	57–35	1.3165	0.0095	73–77	61–71	1.3128	0.0196	56–65	56–65

P (parent), HL (hemolymphagy = fed on cockroaches), HM (hematophagy = fed on mice), O1 (first offspring), O2 (second offspring), O3 (third offspring), % Classif. (% classification), and * statistically significant difference, $p < 0.0024$.

difficult to support the hypothesis of reduced dimorphism proposed by Dujardin et al. [22] in insects under stable breeding conditions as in a laboratory. This result differs from previous observations made in other groups of insects in which the increase in the degree of SSD is generally accompanied by an increase in the body size of females [39]. In our study of *B. ferroae,* the female's wing size, which might be considered as a global estimation of body size, did not vary significantly from one generation to the next (see Table 2) in spite of the appearance of sexual dimorphism. Therefore it is reasonable to suppose that other factors, different from those traditionally used to explain the presence or degree of SSD, could be operating in this species, as has been proposed [38] in Trichoptera. These results demonstrate the need for a cautious interpretation of changes in SSD of laboratory offspring as an analog for domiciliation in Triatominae.

4.2. Shape Variation and Allometry. The choice of a host affects morphophysiological aspects of the Triatominae [9, 40], as well as reproductive and demographic parameters that determine fitness, including development times [2, 27], mortality, and fecundity rates [41–44].

Recently, the host (pigeon and guinea pig) was demonstrated to influence head shape in *T. infestans* [11]. In general terms, shape shows greater stability than size under the influence of certain environmental factors [45]. For example, in the triatomine *R. pallescens*, density and host availability modified the size of wings and heads, but not their shape [14].

In *B. ferroae*, wing data revealed that size contributed significantly to variation of shape (allometric effect). Thus, differences in shape cannot be attributed principally to this effect because the contribution, although significant, is small here and in other species [10, 11, 46]. If an important percentage of the variation of shape is independent of size, then other factors must be involved in the differences of wing shape between groups. In this study, insects were derived from a single population, and environmental factors such as temperature, relative humidity, photoperiod, and density were controlled. Therefore, it is possible that differences in shape are related to the host. In this sense, the results obtained in the analysis of wing shape (free allometry) of male offspring are interesting, where significant differences between the shapes of the generations fed on different hosts (cockroaches versus rodents) appear. This observation confirms that the previously observed shape differences were not simply allometry and supports the hypothesis of host effect on wing morphology (Table 3).

Several studies have shown the influence of host or diet on the induction of morphological changes. Andersson et al. [47] showed that in the carp *Carassius carassius* changes in body shape were induced by diet (zooplankton or benthic chironomids). In *Drosophila* (*repleta* group), shape of the aedeagus changes depending on the cactus host [48].

Here, in *B. ferroae*, the canonical analysis of the variance showed that the Mahalanobis distances were always greater and significant between the phenotype of parental wing shape and that of descendants fed on mice, whether female (2.239–2.461) or male (2.658–2.863). On the contrary, the Mahalanobis distances between the parental phenotype and that of the descendant insects fed on cockroaches were always lesser in the females (1.0127–1.5354), with significant differences only after the third generation and in all the males (0.8661–1.3038), without any statistically significant differences among them for the three laboratory generations. These results indicate a greater similarity in wing shape between parents and offspring raised with cockroaches.

Our results imply that the parental population had fed on invertebrate hosts similar to those used in here and support the viability of the life cycle of *B. ferroae* with cockroaches in natural conditions (human dwellings). These data, coupled with the information relative to the alimentary profile of *B. ferroae* captured in houses that showed that 89.6% of the intestinal contents had hemolymph, probably from cockroaches [4], and with the life history analysis that showed a greater demographic fitness with cockroaches [2], constitute ecological, demographical, and morphological evidence of adaptation of this species to invertebrate hosts.

In addition, the superimposition of the wing shape of females fed on cockroaches produced a greater degree of deformation to the parental configuration than that of males (see Figure 4). As a cause to such deformation, we propose that in the field females may be more eclectic in their diet than males due to different nutritional requirements of the sexes. The simultaneous detection of hemolymph and mammal blood (human and rodent), in the intestinal contents of *B. ferroae* captured in human dwellings, supports this possibility [4].

Furthermore, although the association of *B. ferroae* with vertebrate hosts does not appear to be common under natural conditions (dwellings) and in laboratory conditions showed a low fitness [2], this association could be vital on those occasions where the arthropod host is unavailable. Additionally, feeding on mammal hosts may have relevant epidemiological consequences as it would contribute to the preservation of *T. cruzi*'s cycle in nature, given the susceptibility of *B. ferroae* to infection by this parasite [49].

In *B. ferroae* the wing shapes exhibited by insects fed on mice could be interpreted as the consequence of phenotypic plasticity in insects submitted to stressful conditions, considering the minimally adaptive value (low population growth rate) of such a diet [2].

In conclusion, diverse tendencies demonstrated by the form components, size and shape, suggest that the observed differences may be due to a relative independence of these two aspects of wing morphology [10]. It seems likely that variations in size and shape are influenced by different external variables. For example, size could be more sensitive to small variations in the availability and utilization of resources [50].

Our analysis of phenotypical variation was facilitated by geometric morphometry techniques and made it possible to detect changes in shape produced by a pair of potential hosts (cockroaches and mice) in *B. ferroae*. In addition, the high similarity in wing shape found between parental and offspring fed on cockroaches is consistent with the idea that *B. ferroae* is not an obligate hematophage [2] and that in its spectrum of hosts it prefers arthropods and only occasionally mammals, birds, or reptiles [4].

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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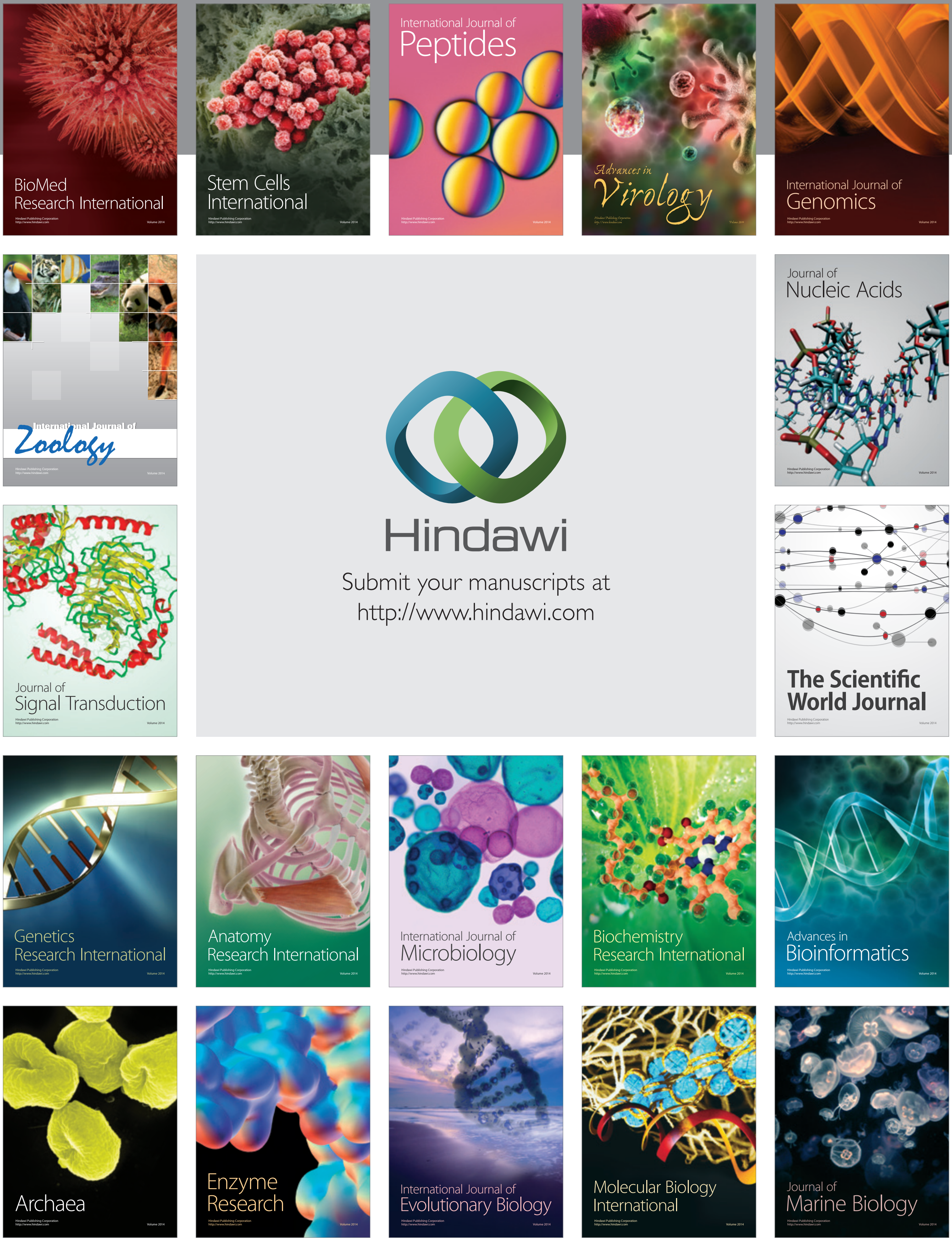
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