



## Growth changes in *Rhodnius pallescens* under simulated domestic and sylvatic conditions

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### ABSTRACT

*Rhodnius pallescens* Barber 1932 is a silvatic species of Triatominae living in palm trees in Colombia and part of Central America. In Colombia, the species did not adapt to domestic structures and is not considered as an important vector for humans. In Panama, Costa Rica and Nicaragua it is a recognized vector adapting to peridomestic and domestic structures.

The main condition required for a Triatominae to be a significant vector of Chagas disease is its ability to colonize human dwellings. The mechanisms of this process are unknown. In this adaptation to domesticity, previous authors have reported a fairly constant reduction in general size and discussed its possible causes in terms of natural selection across various generations or of simple growth changes within one single generation. Some authors suggested that this size change could correspond to the relaxation of a selective pressure in the wild, where larger phenotypes are possibly selected due to their greater capacity to resist temporary food shortages. Others suggested that growth patterns could change quickly in domestic conditions because of faster development time or higher population density.

Up to now, these hypotheses have not been explored experimentally. We built a laboratory protocol measuring the effects of population density and feeding patterns on the growth of *R. pallescens*. A total of 320 first instar nymphs from a 10 generations old laboratory colony were randomly subdivided into four regimes combining density and feeding frequency variation. Using geometric morphometric techniques applied to the emerging adults, we evaluated size and shape variation of the heads and the wings across the four experimental designs. The regimes tentatively paralleled changes occurring for a Triatominae in the transition from silvatic (low population density, low feeding frequency) to domestic (higher density, higher feeding frequency) habitats. Density and feeding frequency combined their effect within the span of one single generation to produce similar size changes for wings and heads, but significant only for the wings. No significant variation could be detected for shape variation, neither for the wing nor for the head.

Our data suggest that selection is not needed to account for observed changes between sylvatic and domestic ecotopes, and they did not agree with an effect of development time on size. They highlighted the importance of the interaction between population density and feeding frequency to produce specific and significant variation in the insect dimensions.

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### 1. Introduction

*Rhodnius pallescens* Barber 1932 is a silvatic species of Triatominae living in palm trees (mainly *Attalea butyracea*) in Colombia and part of Central America. In Colombia, the species sometimes is found in human dwellings, but seems to fail in

establishing definitive colonies (Dujardin et al., 2002); in this country, *R. pallescens* is not considered as an important vector for humans (Guhl et al., 2007). In Panama, Costa Rica and Nicaragua, it is a recognized vector adapting to peridomestic and domestic structures (Christensen and Vasquez, 1981; Calzada et al., 2006; Marin et al., 2006; Zeledón et al., 2006).

The process of adaptation to domestic structures by sylvatic Triatominae is still largely unknown (Schofield et al., 1999). In various species of Triatominae, morphological and, to a lesser extent, genetic changes have been observed associated with their adaptation from silvatic to domestic or laboratory populations

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(Dujardin, 1998). The morphological changes generally involved a reduction in average size (Zeledón et al., 1970; Zeledón, 1981; Dujardin et al., 1997a, 1999; Jaramillo et al., 2002) and a possible increase in size variance (Feliciangeli et al., 2007). The present study focusses on the possible mechanisms of this size variation.

It has been suggested that natural selection may favor larger phenotypes in less favorable (more unstable or “silvatic”) habitats, possibly due to a greater capacity to resist temporary food shortages (Jaramillo et al., 2002; Rodríguez et al., 2007). Alternatively, smaller individuals apparently survive better under laboratory or domestic conditions where food availability is less restricted: as a passive result, the average size would decrease (Schofield, 1996).

Zeledón et al. (1970) and Zeledón (1981) developed the hypothesis that, without necessity of selection, larger size could be attributed to longer development time in silvatic conditions due to delayed moults as produced by incomplete meals. Alternatively, the average size would decrease in domestic conditions because of higher densities altering blood captation due to competition between individuals (Dujardin et al., 1999). In this latter hypothesis, higher densities would be obtained because of the more protected domestic microenvironment relative to the sylvatic one where predators or parasites are more frequent and blood availability less frequent.

We explored here the hypothesis of growth changes within a single generation under controlled experimental conditions: the frequency of feeding, which is supposed to affect size in silvatic conditions, and the crowding, supposed to be an influential factor in domestic conditions.

The mean values of metric variations were compared using classical ANOVA for size (Schlichting and Pigliucci, 1998), and MANCOVA for shape and size (Collyer and Adams, 2007). A special software was written to allow non-parametric tests on inter-individual variance of size, in addition to the study of means, also performing non-parametric tests on interaction between effects.

## 2. Materials and methods

### 2.1. Experimental design

A total of 320 first instar nymphs of *R. pallescens* were selected at random from laboratory colonies that had been maintained for more than 10 generations under controlled conditions of lighting, temperature ( $25 \pm 2^\circ\text{C}$ ), relative humidity ( $80 \pm 5\%$ ) and bimonthly feeding on hens. Nymphs were distributed randomly in four plastic containers of dimensions  $15.6\text{ cm} \times 15\text{ cm} \times 18.3\text{ cm}$ . in a way that two flasks received each one 40 nymphs, and another two received 120 nymphs. Each one of the two containers of 40 and 120 nymphs was fed weekly (40\_W and 120\_W, respectively), another set of two was fed monthly (40\_M and 120\_M, respectively). Nymphs were fed on hens by periods of 35 min until they reached sexual maturity. Hens were maintained according to the criteria of the Research Ethics Committee, based on resolution No. 008430 (1993) of the Colombian Ministry of Health.

### 2.2. Morphometric measurements

Of these 320 nymphs, 211 adults were produced (Table 1). Their head and right wing, dissected and mounted by standard techniques, were photographed with a Nikon 990 digital camera fitted to a Nikon SMS 800 stereomicroscope. The organs were always put at the center of the visual field to reduce the risk of optical distortion. Six landmarks on each right hemi-head and seven on each right wing were selected (Fig. 1). One side only of the body (the right side) was used to avoid interference in the analyses of within individual variation.

**Table 1**

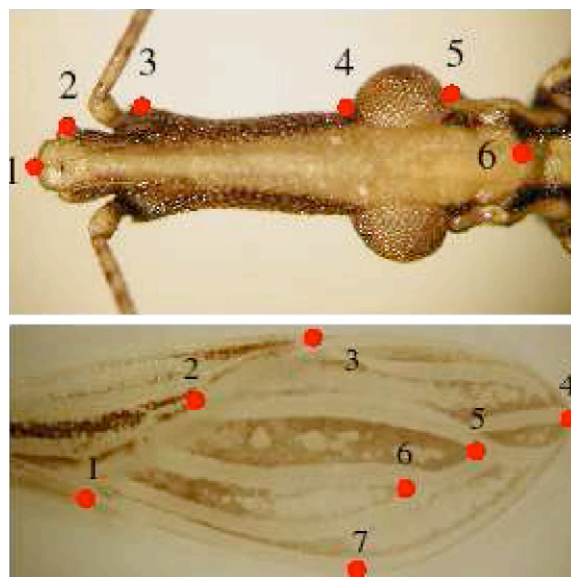
Sample sizes (*n*), mean size (*m*) and standard deviation (S.D.) of each treatment: 40 W = density 40 weekly feeding; 40 M = density 40 monthly feeding; 120 W = density 120 weekly feeding; 120 M = density 120 monthly feeding.

Treatment		Female		Male	
		Wing	Head	Wing	Head
40 W	<i>n</i>	15	17	19	19
	<i>m</i>	947.2	1218.7	893.1	1171.9
	S.D.	46.6	51.1	51.1	50.1
40 M	<i>n</i>	8	11	9	9
	<i>m</i>	1046.0	1234.5	966.3	1180.8
	S.D.	56.5	39.9	52.8	49.8
120 W	<i>n</i>	42	48	46	43
	<i>m</i>	933.3	1217.1	865.3	1159.8
	S.D.	50.2	47.1	61.1	61.6
120 M	<i>n</i>	26	26	35	35
	<i>m</i>	913.7	1212.2	840.2	1154.7
	S.D.	27.1	27.2	25.9	25.4

To detect the effects of possible peripheral optical distortion, we compared two sets of photographs taken on the same set of wings located at different positions in the optical field. The measurement error was estimated by the “repeatability” (*R*) index as described by Arnqvist and Martensson (1998), i.e. a Model II oneway ANOVA on repeated measures, where “*R*” is provided by the ratio of the between individual variance and the total variance.

### 2.3. Geometric morphometrics

For comparing overall wing or head size between groups, we used the isometric estimator known as “centroid size” derived from coordinates data: it is defined 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). Shape variables were obtained through Generalized Procrustes Analysis (GPA) superimposition algorithm and thin-plate spline equation to produce the “partial warps” (Rohlf, 1990; Rohlf and Slice, 1990). Both non-uniform (“partial warps”, strictly speaking) and uniform components were used as shape variable.



**Fig. 1.** Landmarks collected from heads (top) and wings (bottom). The landmarks (LM) are defined as type II (all head LM, wing LM 4 and 7) and type I (remaining wings LM). See (Bookstein, 1990).

Since some samples were small relative to the number of shape variables, we computed their principal components (called “relative warps”, or RW) and used a subset of them without important loss of information (from 77% to 85% of total variation, according to the sample examined). The subsets of first RW were those maximizing the correct classification percentages using cross-validation (Baylac and Frieß, 2005). For the wings, best results were obtained for the first 5 RW in males and 8 RW in females; for the heads, the first 6 RW were found to produce the best reclassifications in both sexes.

## 2.4. Statistical analysis

### 2.4.1. Developmental time and mortality

The developmental time was compared between treatments as the number of adults obtained before 7 months, and after 7 months up to a total duration of 15 months. The  $2 \times 4$  table was submitted to a classical chi square analysis. Mortality was computed as the percentage of dead specimens in each group, whatever the developmental stage (excluding adults).

### 2.4.2. Size

Average sizes according to treatment were plotted to show classical reaction norm graphics (Pigliucci, 2005, 1998). A parametric two-way ANOVA was performed to test for separate and combined effects of population density and feeding frequency (Adams, 2004; Langerhans et al., 2004). A non-parametric approach (see module VAR, <http://www.mpl.ird.fr/morphometrics>) was also applied to allow, in addition to means comparisons, the relevant variances comparisons. This latter estimation of phenotypic plasticity is less frequently considered (Dworkin, 2005), but has been reported for Triatominae (Feliciangeli et al., 2007).

The non-parametric approach performed comparisons of means and variances based on permutations (see columns D1 and D2 of Table 3). At each permutation cycle, individuals were randomly exchanged among groups, the random means and variances were computed and the relevant differences between them (random differences) were scored. A difference was considered significant if the 95% of the random differences was lower than the observed one (99% if the significance level is put at 0.01).

For both means and variance, the same script (VAR) also compared the pair-wise differences (D1 and D2) to test if one pair showed significantly larger or smaller difference than the other one between the same treatments, i.e. the script also tested for interaction between effects (see column D1–D2 of Table 3). To do so, bootstrapping techniques were used as described by Zelditch et al. (2004). At each cycle, each group was sampled with replacement, the pair-wise differences (D1\_40 and D2\_120, or D1\_W and D2\_M, see Table 3) were computed again, and their difference (D1–D2, Table 3) was scored. The 95% confidence interval (c.i.) of these scored differences was computed after 1000 cycles (or the 99% c.i. if the significance level is put at 0.01): if containing zero, the difference between pair-wise differences was considered non-significant. This test for interaction is reported column D1–D2 (see Table 3).

### 2.4.3. Shape

To examine shape variation (Y), we adopted the general approach<sup>1</sup> for the statistical comparison of multivariate vectors of phenotypic change as described by Collyer and Adams (2007).

The procedure is described hereafter, for more details see Collyer and Adams (2007). A two-factor (density, diet) MANCOVA was used to test for differences in head or wing shape and two-factor interaction. Independent variables (X) were the feeding frequencies, population densities, and the centroid size as a covariate, while dependent variables (Y) were the relative warps (RW, i.e. the principal components of the partial warps). A matrix of partial regression coefficients (Bf) was computed using the full design matrix (Xf, including two-factor interaction), and another one (Br) using the reduced design matrix (Xr, excluding two-factor interaction). The least-squares means were computed using the full model (Bf). The phenotypic change vectors were estimated from least-squares means as quantities specified by a magnitude (length) and a direction (angle). It was then possible to compare different phenotypic change vectors by their length<sup>2</sup> differences (D1–D2, see Tables 4 and 5). To evaluate the significance of these values, a residual randomization was performed using the reduced model (Xr). The predicted values ( $Y_r = X_r B_r$ ) and residuals (e) were determined. Residuals were randomized and added to predicted values to produce random values ( $Y^* = Y_r + e$ ) such that non-targeted effects were held constant. The full model was then used to calculate predicted values from the random data, and the values (D1, D2, the difference D1–D2 and the angle between D1 and D2, see Tables 4 and 5) were determined. This procedure was repeated 1000 times to compare the observed values to a distribution of random values (Collyer and Adams, 2007; Collyer et al., 2007).

In a second run, exactly the same analyses were performed but using allometry-free shape variation, i.e. the RW were predicted forcing centroid size to be the same (the grand mean) for all individuals across groups. This procedure was allowed since the common allometric model was not rejected, i.e. the MANCOVA of shape versus size, groups and the corresponding interaction did not show statistical significance for the interaction “size  $\times$  groups” (details not shown).

### 2.4.4. Software

The *tpsDig* (Rohlf, 1992) was used for digitizing landmarks, VAR was used to perform non-parametric ANOVA, COV to compute relative warps and MANCOVA and PAD to compute cross-validated classifications. These scripts can be found at <http://life.bio.sunysb.edu/morph/> and at <http://www.mpl.ird.fr/morphometrics>. The commercial program STATA (Hamilton, 1993) was used to perform ANOVA and to graph reaction norms.

## 3. Results

The individuals grown with higher availability of food and at higher density, a condition which could parallel domestic or laboratory ones, exhibited a faster time of development and a smaller size mean, while those grown with lower availability of food in uncrowded conditions, a condition reflecting what occurs in silvatic habitats, exhibited a slower time of development and a higher size mean.

### 3.1. Repeatability (R)

Comparison of two repeated sets of photographs of the same set of wings showed fairly good agreement for the centroid size ( $R = 0.99$ ), as well as for the very first relative warps (RW1, RW2 and RW3, with  $R = 0.99, 0.89$  and  $0.78$ , respectively) representing most of the shape variation. As observed by Arnqvist and Mårtensson (1998) and by us, the measurement error was increasing on the following RW.

<sup>1</sup>  $Y = XB + e$ , where X is an  $n \times k$  design matrix describing the k model effects for n objects, B is a  $k \times p$  matrix of partial regression coefficients for p response variables, and e is the  $n \times p$  matrix of residuals.

<sup>2</sup> The length is measured as an Euclidean distance between least square means.

**Table 2**

Two-way ANOVA for centroid size, by density and feeding patterns. Density = either 40 or 120; feeding = either weekly or monthly feeding frequency.

Sex	Trait	Density	Feeding	Interaction
Females	Wings	0.000*	0.001*	0.000*
	Heads	0.227	0.580	0.293
Males	Wings	0.000*	0.039	0.000*
	Heads	0.093	0.853	0.555

\* Significant interaction after Bonferroni adjustment at alpha 0.05.

### 3.2. Developmental time and mortality

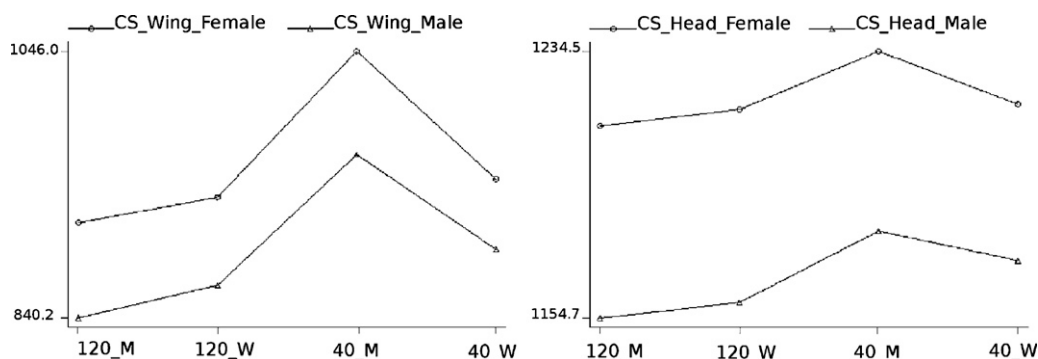
The 320 nymphs produced a total of 211 adults. Among the latter, all the weekly fed specimens ( $n = 130$ ), versus only 42 (50%) of the monthly fed ones, arrived to adults before the seventh month. The remaining monthly fed nymphs ( $n = 39$ ) required an additional 7 months to reach adult stage. These differences were statistically significant ( $P < 0.001$ , details not shown). The percentage of dead specimens (at various stages of development, excluding adults) ranged from 10% to 22% in the weekly fed groups, while it was 49% and 50% in the monthly fed ones.

### 3.3. Size

Sample sizes, means and standard deviations of the four treatments are listed in Table 1. In general, the individuals grown at a higher population density exhibited smaller sizes for wings and heads (Figs. 3 and 4), but significant changes were observed for wings only (Tables 2 and 3). The metric changes followed approximately the same trends in males and females (Fig. 2).

The analyses on size variation used both parametric (Table 2) and non-parametric (Table 3) tests to compare means and test for interaction between effects, while the comparisons of variances used non-parametric tests only (Table 3). Both approaches examining the means produced similar results, and for either the mean or the variance changes, they highlighted the importance of the interaction between population density and feeding frequency. In addition to parallel the classical ANOVA results, Table 3 showed the observed differences between means according to comparisons.

In both sexes, only wings presented significant changes of mean centroid size induced by either density or feeding frequency. There was a significant interaction between these two factors (Table 2, and see column D1–D2 of Table 3). In males only there was a significant change in the variance of size for both wings and heads, with significant interaction for the wing variation (see bottom part of Table 3).



**Fig. 2.** Reaction norms of wings and heads size. 40\_W, density 40 when weekly fed; 40\_M, density 40 when monthly fed; 120\_W, density 120 when weekly fed; 120\_M, density 120 when monthly fed.

**Table 3**

Non-parametric tests for means and variances of size. D1\_40 = differences of means between weekly and monthly feeding rhythms, at density 40; D2\_120 = the same differences at density 120; D1\_W = differences of means between densities when weekly fed; D2\_M = the same differences when monthly fed; D1\_40 = differences of variances between feeding rhythms, at density 40; D2\_120 = the same differences at density 120; D1\_W = differences of variances between densities when weekly fed; D2\_M = the same differences when monthly fed; D1–D2 = either (D1\_40 - D2\_120) or (D1\_W - D2\_M). Interaction = D1 - D2.

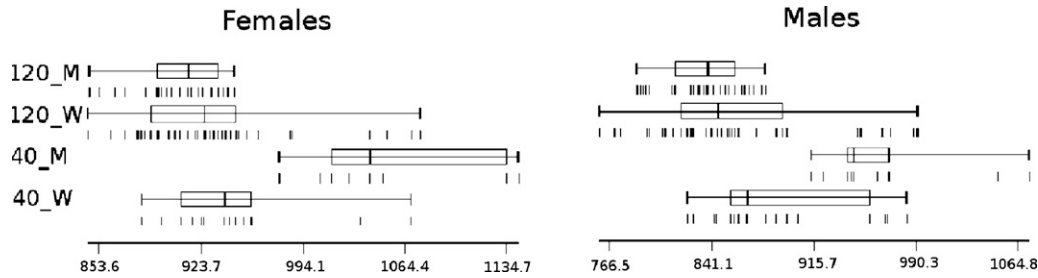
Sex	Trait	Feeding		Density		Interaction
		D1_40	D2_120	D1_W	D2_M	
Mean size						
Females	Wings	-98.8*	19.7	13.9	132.4*	-118.5*
	Heads	-15.8	4.9	1.6	22.3	-20.7
Males	Wings	-73.3*	25.1	27.7	126.1*	-98.4*
	Heads	-8.9	4.6	12.5	26.0	-13.5
Variance of size						
Females	Wings	-1027.6	1787.5	-352.3	2462.8	-2815.1
	Heads	1017.0	1483.1	393.3	859.4	-466.1
Males	Wings	-172.7	3057.2*	-1115.1	2114.8	-3229.9*
	Heads	31.1	3141.9	-1276.7	1834.1	-3110.8

\* Significant interaction after Bonferroni adjustment at alpha 0.05.

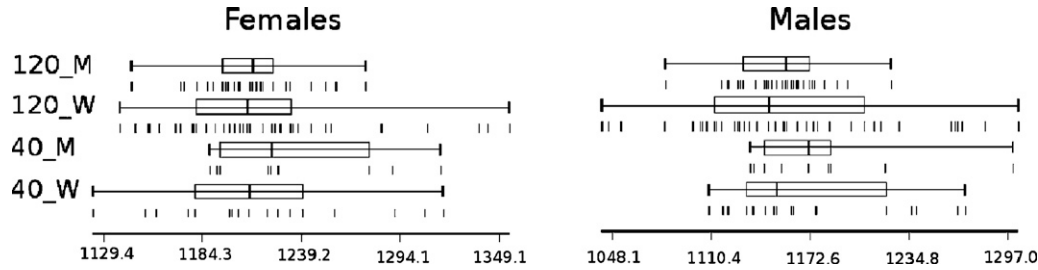
The lower feeding frequency produced a significant increase of wing size in males and females only when at low density (Table 3, see column D1\_40, and Fig. 3); the lower population density produced a significant increase of wing size in both sexes only at a monthly feeding frequency (Table 3, see column D2\_M, and Fig. 2 or 3). The D1–D2 column of Table 3 corresponds to a non-parametric test of interaction: it indicates that the observed changes of size between insects submitted to different diets were significantly more important at one density (40\_W and 40\_M) than at the other one (120\_W and 120\_M); alternatively, it indicates that the observed changes between densities were significantly more important at one feeding frequency (40\_M and 120\_M) than at the other one (40\_W and 120\_W).

Table 3 also produces a similar set of comparisons and presentation for variance of size (see its bottom part). Variance of size was generally similar among treatments. It could change however between feeding frequencies at high population density: the 120\_W treatment showed larger range of sizes than the 120\_M one (Figs. 3 and 4), and this was significant in males for both heads and wings (Table 3). In the changes of size variance for males there was an interaction between density and feeding frequency: the change at high density was significantly more important than at lower density (D1–D2).

When looking at the range of sizes exhibited by each group (Figs. 3 and 4), an interesting visual pattern could be observed: the



**Fig. 3.** Median and dispersion of wings size in each treatment. Each box shows the group median separating the 25th and 75th quartiles, with the 10th and 90th quartiles shown as lines on each side of the box. 40\_W, density 40 when weekly fed; 40\_M, density 40 when monthly fed; 120\_W, density 120 when weekly fed; 120\_M, density 120 when monthly fed.



**Fig. 4.** Median and dispersion of head size in each treatment. Each box shows the group median separating the 25th and 75th quartiles, with the 10th and 90th quartiles shown as lines on each side of the box. 40\_W, density 40 when weekly fed; 40\_M, density 40 when monthly fed; 120\_W, density 120 when weekly fed; 120\_M, density 120 when monthly fed.

range of size seemed to be amputated in the area of large-sized individuals at high density (120\_M), while only small-sized individuals seemed to have disappeared at low population density (40\_M).

### 3.4. Shape

No significant difference was disclosed for shape changes, nor was it for allometry-free shape changes, as measured by differences in magnitudes of changes (D1–D2, see Tables 4 and 5) or by the angles, neither for separate factors effects (density, feeding frequency) nor for their interaction (Tables 4 and 5). However, even for allometry-free differences between treatments, shape changes in both sexes followed the same general pattern as size changes, i.e. the distances between treatments disclosed higher values for wings than for head.

**Table 4**

Magnitude and direction of phenotypic changes in female specimens of *R. palllescens*. E = Euclidean distance between relative warps; Ec = Euclidean distance between relative warps corrected for size variation; D1\_40 = distances E and Ec between feeding rhythms at density 40; D2\_120 = the same distances at density 120; D1\_W = distances between densities when weekly fed; D2\_M = the same distances when monthly fed; D1–D2 = (D1\_40–D2\_120) or (D1\_W–D2\_M). Angle = angle between D1 and D2. W = wing; H = head. No angle was significant. All distances and or differences were not significant.

Females		Wing		Head	
		E	Ec	E	Ec
Interaction		NS	NS	NS	NS
Feeding	D1_40	0.013	0.021	0.012	0.011
	D2_120	0.013	0.012	0.004	0.004
	D1–D2	0.000	0.009	0.008	0.007
Angle			80	17	
Density	D1_W	0.013	0.013	0.007	0.006
	D1_M	0.022	0.027	0.006	0.005
	D1–D2	–0.009	–0.014	0.001	0.001
Angle		57	80		

## 4. Discussion

A general reduction in body size had been noted in the laboratory for Triatominae reared over successive generations (Szumlewicz, 1976; Zeledón, 1981; Zeledón et al., 1970). Various studies also had shown that in the domestic habitat specimens also tended to be smaller than their silvatic conspecifics (Harry, 1994; Dujardin et al., 1997a,b, 1999; Jaramillo et al., 2002). In the literature on Triatominae, the hypotheses raised until now accounting for these differences in size could be subdivided into the “selection hypothesis” and the “growth hypothesis”.

The selection hypothesis was formulated for both environments, domestic and silvatic, and mainly related to availability of food. Because domestic conditions are apparently advantageous (higher and more regular feeding frequency, absence of predators), improved survivorship of small specimens could be expected in

**Table 5**

Magnitude and direction of phenotypic changes in male specimens of *R. palllescens*. E = Euclidean distance between relative warps; Ec = Euclidean distance between relative warps corrected for size variation; D1\_40 = distances E and Ec between feeding rhythms at density 40; D2\_120 = the same distances at density 120; D1\_W = distances between densities when weekly fed; D2\_M = the same distance when monthly fed; D1–D2 = (D1\_40–D2\_120) or (D1\_W–D2\_M). Angle = angle between D1 and D2. W = wing; H = head. No angle was significant. All distances and or differences were not significant.

Males		Wing		Head	
		E	Ec	E	Ec
Interaction		NS	NS	NS	NS
Feeding	D1_40	0.019	0.024	0.007	0.007
	D2_120	0.020	0.016	0.007	0.008
	D1–D2	–0.001	0.008	0.000	–0.001
Angle			51	45	
Density	D1_W	0.012	0.014	0.003	0.002
	D1_M	0.019	0.018	0.005	0.005
	D1–D2	–0.007	–0.004	–0.002	–0.003
Angle		70	69		

domestic conditions, and average size would decrease (Schofield, 1996). Alternatively, larger specimens should be favored in sylvatic conditions of life due to their greater capacity to resist temporary food shortages (Jaramillo et al., 2002; Rodríguez et al., 2007).

Simple growth changes were also suggested. Zeledón et al. (1970) and Zeledón (1981) supposed that the larger size of sylvatic specimens could be attributed to longer development time in silvatic conditions due to delayed moults as produced by incomplete meals. Dujardin et al. (1999) considered that the average size would decrease in domestic conditions because of higher population densities altering blood captation due to competition between individuals.

The present study examines the simple growth change hypothesis. It provides a quantitative analysis of metric variation, development time and mortality under controlled changes in population density and feeding frequency.

Mortality was related to the feeding frequency, without apparent interaction of density. Indeed, from weekly to monthly frequency of feeding, mortality increased more (or much more) than twice. An obvious relationship was evidenced also between feeding frequency and development time. The more fed, the faster developed, whatever the population density.

Our data did not support an explanation involving the effect of longer developmental cycle on size (Zeledón, 1981; Zeledón et al., 1970). In our experiments, there was no detectable correlation of development time with size. Such relationship however is commonly reported for other insects—although not always a positive correlation (Klingenberg and Spence, 1997).

Size was always lower at higher densities, whatever the feeding frequency, which suggests the primary importance of population density. However, density and feeding frequency clearly interacted to produce specific and significant changes in the insect dimensions. For instance, the direction of size change from the weekly feeding condition to the monthly one was depending on the population density: an increase at low density, a decrease at high density.

In our experiments, two treatments could parallel known conditions of life for silvatic and domestic specimens: 40\_M and 120\_W, respectively. A low population density and a low feeding frequency is supposed to occur in silvatic habitat (40\_M), while a (much) higher population density and feeding frequency are commonly observed in domestic conditions (120\_W). The metric changes disclosed between these two treatments (see Fig. 3) were indeed similar to those reported by previous authors: a reduction in mean size (Dujardin et al., 1999; Jaramillo et al., 2002; Rodríguez et al., 2007) together with an increase of variance (Felicangeli et al., 2007), although this latter was not constantly reported or significant, depending on the organ (Jaramillo et al., 2002) or the species of Triatominae (Rodríguez et al., 2007). In our results, significant increase of variance was observed in males only (Table 3).

According to our data, no other factor is required than the combination of high population density and high feeding frequency to reproduce the observed changes in insect dimensions in their transition from silvatic to domestic habitats. This conclusion supports the “growth hypothesis”, but does not reject the “selection hypothesis”. Selection is likely, but it would need a laboratory protocol conducted over various generations to be detected. Although very speculative, the simple visual inspection of size ranges according to treatments reveals a possible non-random mortality of nymphs. Indeed, there is an apparent lack of small sized individuals at low population density, and an apparent lack of large sized individuals at high density (see Fig. 3). This pattern could suggest selective mortality at low feeding conditions, where mortality rate was as high as 50%. Thus, not only more

individuals would die at low feeding frequency, but actually a defined category of size according to population density.

Our study depicted metric changes as they may occur within one single generation only. Characters able to change as fast as the changing environment may be called “labile” characters (Scheiner, 1993), and are considered as important in phenotypic evolution (West-Eberhard, 1989). In agreement with the common observation of size being more labile than shape, the significant changes recorded here were exclusively size modifications. The lack of significant shape or allometry-free shape variation in both wings and heads was in agreement with the common idea of shape as a more stable character (than size) in the face of environmental perturbations. It could however be attributed also to some lack of statistical power since some of our samples were relatively small (ranging from 8 to 48 specimens), restricting the total amount of shape usable for comparison. Nevertheless, results did not change when using the total number of relative warps (RW). The lack of significant differences between rearing conditions on shape alone is not likely to be related to excess of noise as an effect of possible optical distortion, since we used the first RW where high repeatability scores were obtained while we did not use the last ones, where repeatability was consistently decreasing (Arnqvist and Mårtensson, 1998)(see Section 2).

Significant mean size variation was limited to the wings. There was no such change detected for the head size. Because heads showed similar trends than wings (Fig. 2), this lack of significance was probably due to either low statistical power, lower quality of landmarks (all head landmarks are type II landmarks of see Fig. 1), or both. They also could indicate a better canalization for head than for wing dimensions. However, both wings and heads showed significant changes for the variance of size, limited to males only (Table 3).

Thus, as long as *R. pallens* is a representative species of the Triatominae, our results suggest that no additional factor is needed than the two factors (density and feeding) explored here to account for commonly observed differences between silvatic and domestic Triatominae. This is not to say that we can exclude the intervention of possible complementary forces. For instance, selective mortality of small specimens in silvatic conditions could be enhanced by the activity of predators, which are generally absent or less frequent in domestic habitat.

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## References

- Adams, D.C., 2004. Character displacement via aggressive interference in Appalachian salamanders. *Ecology* 85, 2664–2670.
- Arnqvist, G., Mårtensson, T., 1998. Measurement error in geometric morphometrics: empirical strategies to assess and reduce its impact on measure of shape. *Acta Zool. Acad. Sci. Hung.* 44 (1/2), 73–96.
- Baylac, M., Frieß, M., 2005. Fourier descriptors, Procrustes superimposition, and data dimensionality: an example of cranial shape analysis in modern human populations. In: Slice (Eds.), *Modern Morphometrics in Physical Anthropology*. (Chapter 6), pp. 145–165.
- Bookstein, F.L., 1990. Introduction to methods for landmark data. In: Rohlf, F.J., Bookstein, F.L. (Eds.), *Proceedings, Michigan Morphometrics Workshop*, 1988. The University of Michigan Museum of Zoology, Special Publication No. 2, Ann Arbor, MI, pp. 216–225.
- Bookstein, F.L., 1991. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press, Cambridge, pp. 435.
- Calzada, J.E., Pineda, V., Montalvo, E., Alvarez, D., Santamaria, A.M., Samudio, F., Bayard, V., Caceres, L., Saldaña, A., 2006. Human trypanosome infection and the

- presence of intradomicile *Rhodnius pallescens* in the western border of the Panamá canal, Panamá. *Am. J. Trop. Med. Hyg.* 74 (5), 762–765.
- Christensen, H.A., de Vasquez, A.M., 1981. Host feeding profiles of *Rhodnius pallescens* (Hemiptera: Reduviidae) in rural villages of Central Panamá. *Am. J. Trop. Med. Hyg.* 30 (1), 278–283.
- Collyer, M., Adams, D.C., 2007. Analysis of two state multivariate phenotypic change in ecological studies. *Ecology* 88, 683–692.
- Collyer, M., Stockwell, C.A., Adams, D.C., Hildegard, M., 2007. Phenotypic plasticity and contemporary evolution in introduced populations: evidence from translocated populations of white sands pupfish (*Cyprinodon tularosa*). *Ecol. Res.* 22, 902–910.
- Dujardin, J.P., 1998. Population genetics and the natural history of domestication in Triatominae. *Mem. Inst. O. Cruz* 93 (Suppl. II), 34–36.
- Dujardin, J.P., Bermudez, H., Casini, C., Schofield, C.J., Tibayrenc, M., 1997a. Metric differences between silvatic and domestic *Triatoma infestans* (Hemiptera, Reduviidae) in Bolivia. *J. Med. Entomol.* 34 (5), 544–552.
- Dujardin, J.P., Bermúdez, H., Schofield, C.J., 1997b. The use of morphometrics in entomological surveillance of silvatic foci of *Triatoma infestans* in Bolivia. *Acta Trop.* 66, 145–153.
- Dujardin, J.P., Schofield, C.J., Panzera, F., 2002. Los Vectores de la Enfermedad de Chagas. Investigaciones taxonómicas, biológicas y genéticas. *Académie Royale des Sciences d’Outre-Mer, Classe des Sciences naturelles et médicales. Traduction espagnole.*
- Dujardin, J.P., Steindel, M., Chavez, T., Martínez, E., Schofield, C.J., 1999. Changes in the sexual dimorphism of Triatominae in the transition from natural to artificial habitats. *Mem. Inst. O. Cruz* 94, 565–569.
- Dworkin, I., 2005. Canalization, cryptic variation and developmental buffering: a critical examination and analytical perspective. In: Hallgrímsson, B., Hallgrímsson, B.K., Hall, B.K. (Eds.), *Variation*. Academic Press, (Chapter 8), pp. 131–158.
- Feliciangeli, M., Sanchez-Martin, M., Marrero, R., Davies, C., Dujardin, J., 2007. Morphometric evidence for a possible role of *Rhodnius prolixus* from palm trees in house re-infestation in the State of Barinas (Venezuela). *Acta Trop.* 101, 169–177.
- Guhl, F., Aguilera, G., Pinto, N., Vergara, D., 2007. Actualización de la distribución geográfica y ecoepidemiología de la fauna de triatominos (Reduviidae: Triatominae) en Colombia. *Biomédica* 27 (Suppl. 1), 143–162.
- Hamilton, L.C., 1993. *Statistics with Stata 3*. Duxbury Press, Wadsworth, Belmont, CA.
- Harry, M., 1994. Morphometric variability in the Chagas’ disease vector *Rhodnius prolixus*. *Jpn. J. Genet.* 69, 233–250.
- Jaramillo, N., Castillo, D., Wolff, M., 2002. Geometric morphometric differences between *Panstrongylus geniculatus* from field and laboratory. *Mem. Inst. O. Cruz* 97 (5), 667–673.
- Klingenberg, C.P., Spence, J.R., 1997. On the role of body size for life-history evolution. *Ecol. Entomol.* 22, 55–68.
- Langerhans, R.B., Layman, C.A., Shokrollahi, A.M., DeWitt, T.J., 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution* 58, 2305–2318.
- Marin, F., Lugo, E., Valle, S., Zeledón, R., 2006. Notes on *Rhodnius pallescens*, *Triatoma ryckmani* and four other species of triatomines from Nicaragua. *Ann. Trop. Med. Parasitol.* 100 (6), 181–186.
- Pigliucci, M., 1998. Ecological and evolutionary genetics of *Arabidopsis*. *Trends Plant Sci.* 3, 485–489.
- Pigliucci, M., 2005. Evolution of phenotypic plasticity: where are we going now? *Trends Ecol. Evol.* 20, 481–486.
- Rodríguez, J., González, O., Nodarze, J., Fidalgo, L., Dujardin, J., 2007. Morphometric changes of *Triatoma flavida* Neiva, 1911 (Hemiptera: Triatominae) in the transition from sylvatic to laboratory condition. *Rev. Inst. Med. Trop. São Paulo* 49, 127–130.
- Rohlf, F.J., 1990. Rotational fit (Procrustes) methods. In: Rohlf, F., Bookstein, F. (Eds.), *Proceedings of the Michigan Morphometrics Workshop. Special Publication Number 2*. The University of Michigan Museum of Zoology, Ann Arbor, MI, pp. 227–236, 380.
- Rohlf, F.J., 1992. *Tpsdig: A Program for Digitizing Landmarks*. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY.
- Rohlf, F.J., Slice, D.E., 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool.* 39 (40), 59.
- Scheiner, S.M., 1993. Genetics and evolution of phenotypic plasticity. *Ann. Rev. Ecol. Syst.* 24, 35–68.
- Schlichting, C.D., Pigliucci, M., 1998. *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer Associates, Inc., Sunderland, MA, 387 pp.
- Schofield, C.J., 1996. Overview—biosystematics of the Reduviidae. In: *Proceedings of the International Workshop on Population Genetics and Control of Triatominae Santo Domingo de los Colorados. Ecuador*, pp. 37–42.
- Schofield, C.J., Diotaiuti, L., Dujardin, J.P., 1999. The process of domestication in Triatominae. *Mem. Inst. O. Cruz* 94 (Suppl. I), 375–378.
- Szumlewicz, A.P., 1976. Laboratory colonies of Triatominae, biology and population dynamics. In: *American Trypanosomiasis Research. PAHO Scientific Publication 318*, PAHO Washington, DC, pp. 63–82.
- West-Eberhard, M., 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20, 249–278.
- Zelditch, M.L., Swiderski, D.L., D., S.H., Fink, W.L., 2004. *Geometric Morphometrics for Biologists: A primer*. Elsevier, Academic Press, New York.
- Zeledón, R., 1981. El *Triatoma dimidiata* (Latreille 1811) y su relación con la enfermedad de Chagas. Editado por INCIENSA y Editorial EUNED, 164 pp.
- Zeledón, R., Calvo, N., Marin, F., Lugo, E., Valle, S., 2006. Distribution and ecological aspects of *Rhodnius pallescens* in Costa Rica and Nicaragua and their epidemiological implications. *Mem. Inst. O. Cruz* 101 (1), 75–79.
- Zeledón, R., Guardia, V.M., Niga, A.Z., Swartzwelder, J.C., 1970. Biology and ethology of *Triatoma dimidiata* (Latreille, 1811). I. Life cycle, amount of blood ingested, resistance of starvation, and size of adults. *J. Med. Entomol.* 7, 313–319.