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Morphometrical changes in eggs of *Rhodnius prolixus* (Heteroptera: Reduviidae) during development

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Abstract

CHAVES LF, RAMONI-PERAZZI P, LIZANO E, AÑEZ N. 2003. Morphometrical changes in eggs of *Rhodnius prolixus* (Heteroptera: Reduviidae) during development. Entomotropica 18(2):83-88.

We explore morphometric changes during development in 124 eggs of *Rhodnius prolixus*, kept under laboratory conditions. Measurements were performed using a dissecting microscope coupled to a video camera connected to a monitor. Our results show that only maximal diameter varies significantly ($t = 14.61$, d.f. = 123, $P < 1{,}19*10^{-18}$) changing from (0.88 ± 0.05) mm to (0.93 ± 0.04) mm. Such changes are correlated in a positive way according to a linear function ($R^2 = 0.63$; $F = 206.29$; d.f.= 1, 121; $P<0.0001$). The increase of this measurement is mainly explained by the intercept value ($A = 0.186 \pm 0.052$; $t = 3.57$; d.f.= 120; $P < 5*10^{-4}$), and this value could represent the maximun elasticity of eggs in this species.

Additional key words: Development, eggs, *Rhodnius prolixus*, Triatominae.

Resumen

CHAVES LF, RAMONI-PERAZZI P, LIZANO E, AÑEZ N. 2003. Cambios morfométricos en huevos de *Rhodnius prolixus* (Heteroptera: Reduviidae) durante su desarrollo. Entomotropica 18(2):83-88.

Exploramos los cambios morfométricos durante el desarrollo de 124 huevos de *Rhodnius prolixus*, mantenidos bajo condiciones de laboratorio. Las medidas fueron realizadas empleando un microscopio de disección acoplado a una cámara de video conectada a un monitor. Nuestros resultados indican que sólo el diámetro máximo aumenta de manera significativa ($t = 14{,}61$, d.f.= 123, $P < 1{,}19*10^{-18}$) pasando de (0,88 ± 0,05) mm a (0,93 ± 0,04) mm. Tales cambios están correlacionados de una manera positiva de acuerdo a una función lineal ($R^2 = 0{,}63$; $F = 206{,}29$; d.f.= 1, 120; $P<0{,}0001$). El aumento en las medidas es explicado principalmente por el valor del intercepto ($A = 0{,}186 \pm 0{,}052$; $t = 3{,}57$; d.f.= 120; $P < 5*10^{-4}$), dicho valor podría representar la elasticidad máxima de los huevos de esta especie.

Palabras clave adicionales: Desarrollo, huevos, *Rhodnius prolixus*, Triatominae.

Introduction

Rhodnius prolixus, Stål (1859), is in northern South America the main natural vector of *Trypanosoma cruzi*, Chagas (1909), which causes Chagas disease (Schofield & Dujardin 1997). In the World an estimation for a total eradication and control of vectorial transmission is for the year 2010 (Visschedijk & Siméant 1998). This proposition appears to be very optimistic considering recent reports on domiciliary invasions by Triatominae species, where they have been previously eradicated (Almeida et al. 2000). Such situation points out to the importance and relevance of further studies of the natural history of these insects, especially those poorly understood stages of their life cycle, as may be eggs (Barata 1998).

Information on eggs measurements have been previously produced by researchers interested in the biology of *R. prolixus*. Brumpt (1913) indicates a length(L) of 2 mm; Uribe (1925) shows a L of 2.5 mm; Briceño-Iragorry (1934) recorded a L of 1.95 mm, a diameter(D) of 0.80 mm, and a caudal to neck length of 1.85 mm; Galliard (1935) reports a L among 1.7 and 1.8 mm and a D of 0.820 mm; Barata et al. (1980) reported a L of (1.782 ± 0.067)mm and a D of (0.880 ± 0.044)mm. None of these works were done

considering the possibility that measures change along time.

In this paper we explore if morphometrical changes can occur in areas *Rhodnius prolixus* eggs, and if changes occur in areas whose exochorion cells have a geometry that allow these changes; based on the results of a paper by Chaves & Añez (2003).

Materials and Methods

A total of eighteen couples of adult *R. prolixus* from the same cohort were used. The Triatomine bugs were obtained from a closed colony kept at the "Herman Lent" entomology lab, Facultad de Ciencias, Universidad de Los Andes, Mérida, Venezuela. Bugs were fed to engorgement once, directly on a restrained chicken and then kept on cylindrical vials 5 cm in length and 5 cm in diameter with a piece of paper. They were maintained at 25 °C, 70-75% RH, and 12:12(L:D) photoperiod. The laid eggs were daily removed from the vials. Identified eggs were horizontally fixed on plates covered with a double side adhesive tape 3M®, trying to maintain the same orientation as they were oviposited. A daily morphometrical record was made using a dissecting microscope coupled to a video system. Once all eggs hatched, for each egg, the records of the oviposition day (hereafter O) and the day before hatching (hereafter BHD) were measured using a graduate monitor with 120:1 scale, and a rule of 1 mm appreciation. Figure 1 shows the four morphometric variables considered during the study, they were chosen based on the measures described by as happens in any morphometric study we choose easily identifiable landmarks, as those used by Briceño-Iragorry (1934), as well as the egg's neck diameter. A total of 124 eggs were measured.

A Principal Component Analysis (Norman & Streiner 1996) was performed using Multivariate Statistical Package 3.12 (Kovach Computing Services 2001), considering the measurement at O to explore their correlation level. Measurements were previously standardized and centered. Each trait was compared at O and BHD through a box-plot followed by a paired *t* test (Norman & Streiner 1996). A scatter-plot was performed for each of those measurements whose changes were statistically significant in time. These scatter-plot included a unit slope line, to see the tendency in change and determine which plots should be removed from further linear regression analyses. Finally, a least square linear regression analysis (Norman & Streiner 1996). was performed considering only the chosen plots. All these analyses were performed using Microcal™Origin™ 5.0 (Microcal Software Incorporated 1997).

Results

A 100% of the 124 eggs hatched. Hatching time averaged 18.61 ± 1.20 days (Mean ± S. D.). Table 1 summarizes the values of the four morphometric variables considered at oviposition day and the day before hatching. Maximal diameter was the only measurement that changed in its mean value throughout the development of eggs.

Principal Component Analysis indicates and elevated level of correlation between Nd and Md (r= 0.828), and Nl and Ml (r= 0.800). Figure 2 suggests that only Md varied significantly during the egg development. Statistical significance was corroborated by paired *t* test ($P < 0.05$). Figure 3 shows the scatterplots contrasting Md at O and BHD values, and indicates that only two points are below the unit slope line, which can be interpreted as the eggs increase their Md. Figure 4 represents the fitted regression line obtained by least square method. The ANOVA indicates that correlation is statistically significant ($P < 0.05$). The *t* tests indicates that both slope and intercept are statistically significant ($P < 0.05$).

Discussion

The hatching level observed in our study agrees with Clark (1935) and Noriega (1992). The hatching time observed was similar to those reported in previous works (Buxton 1930; Lent & Valderrama 1977).

Regarding our morphometric measurements, maximal lengths were similar to those previously recorded in the literature (Brumpt 1913; Briceño-Iragorry 1934; Galliard 1935; Barata et al. 1980), excepting Uribe (1927), who recorded a maximal length of 2.5 mm. Maximal diameters were also similar to those previously recorded by other authors (Briceño-Iragorry 1934; Galliard 1935; Barata et al. 1980). Moreover, caudal to neck length is similar to that reported by Briceño-Iragorry (1934). Such coincidences are interesting considering the differences of measurement methods and the origin of the specimens. It indicates stability of such measurements throughout the species range. The remaining morphometric variable surveyed (diameter at egg neck) has not been recorded previously and, thus, we have no comparison reference.

Our results indicate that eggs increase their Md during their development. The two observed exceptions are eggs that were probably mishandled, being placed in a different orientation than that of the parent laying. The

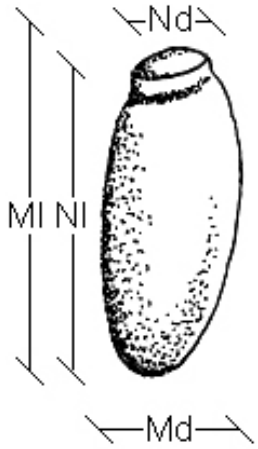
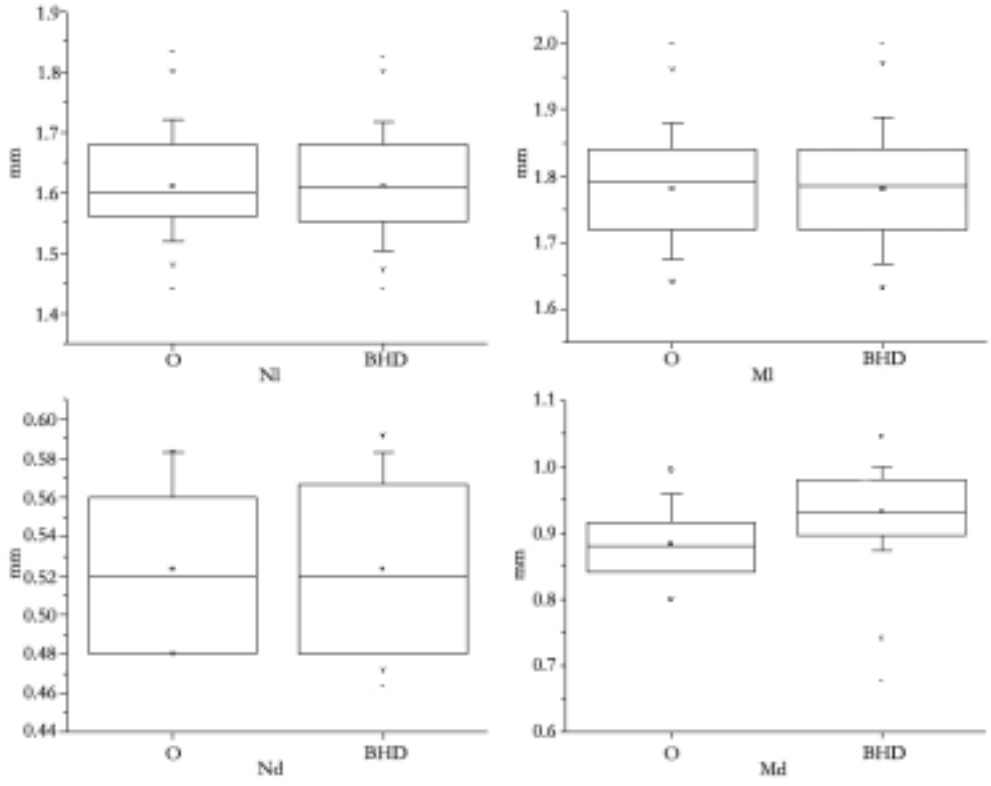


FIGURE 1. Measures: maximal length(Ml), maximal diameter(Md), diameter at egg neck(Nd) and caudal to neck length(Nl).

TABLE 1. Mean values, Standard deviation (S.D.), and ranges of: egg neck diameter (Nd), maximal diameter (Md), caudal to egg neck length (Nl) and maximal length (Ml) of *Rhodnius prolixus*' eggs measured at oviposition day and before hatching day.

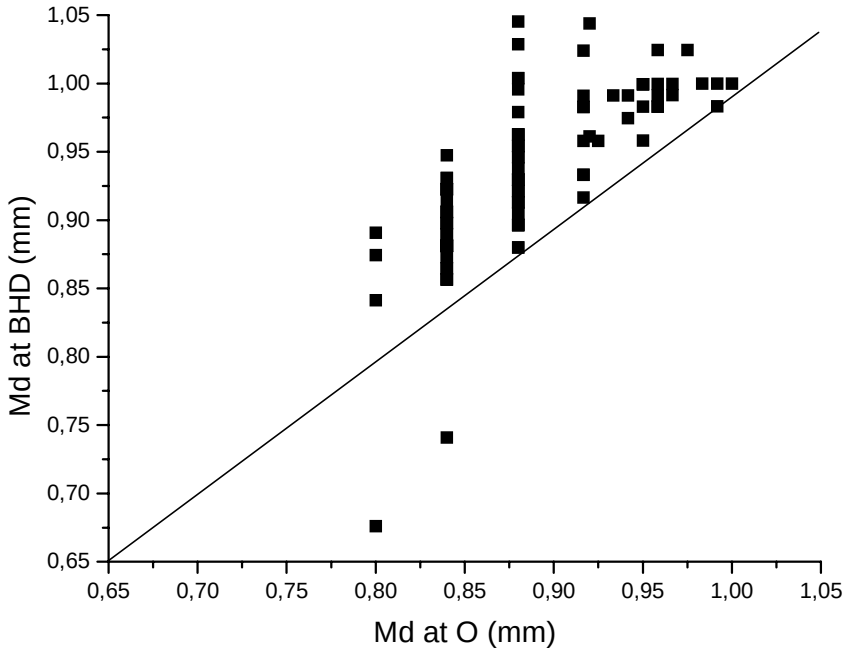
Variable	Oviposition Day		Before Hatching Day	
	Mean ± S.D. (mm)	Range (mm)	Mean ± S.D. (mm)	Range (mm)
Nd	0.52 ± 0.04	0.48 – 0.58	0.52 ± 0.04	0.47 – 0.59
Md	0.88 ± 0.05	0.80 – 1.00	0.93 ± 0.04	0.67 – 1.05
Nl	1.61 ± 0.08	1.44 – 1.83	1.61 ± 0.08	1.44 – 1.83
Ml	1.78 ± 0.08	1.64 – 2.00	1.78 ± 0.08	1.64 – 2.01

FIGURE 2. Box plots of the morphometric egg variables in *Rhodnius prolixus*.



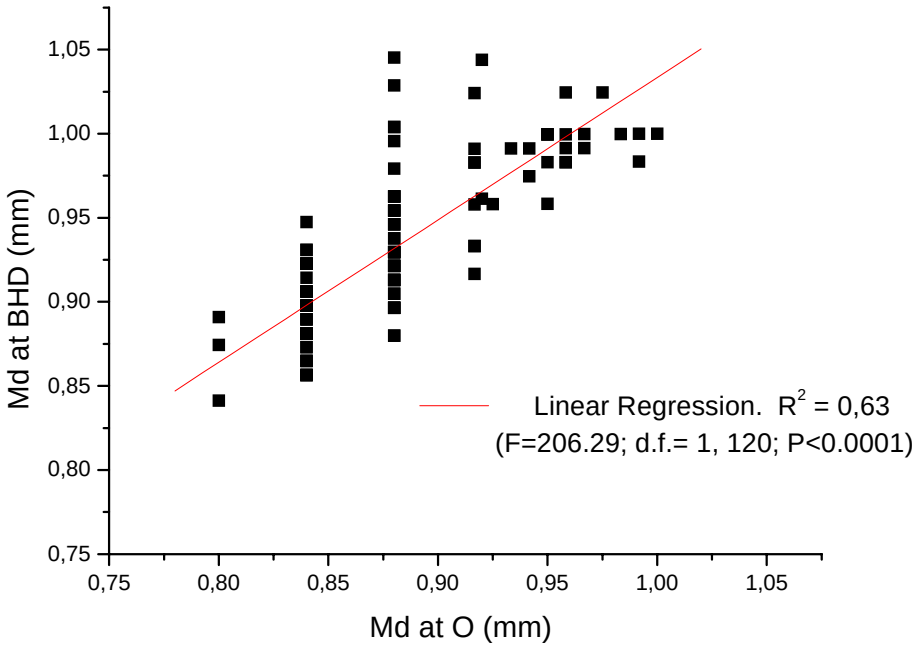
Nl= Caudal to Neck Length (**t=** 0.76, **d.f.=** 123, P < 0.44); **Ml=** Maximal length (**t=** 1.14, **d.f.=** 123, P < 0.25); **Nd=** neck diameter (**t =** 0.11, **d.f.=** 123, P < 0.90); **Md=** Maximal diameter(**t=** 14.61, **d.f.=** 123, P < $1{,}19*10^{-18}$). **O=** oviposition day; **BHD=** before hatching day.

FIGURE 3. Scatterplot contrasting Maximal Diameter (Md) at oviposition day (O) and before hatching day (BHD) values.



The solid line has a unit slope.

FIGURE 4. Fitted regression line obtained by least square method between maximal diameter (Md) at oviposition day (O) and before hatching day (BHD) values.



Linear regression function: $\mathbf{Md_{BHD}}$**=** A + B(Md_O). Md_{BHD} is maximal diameter at before hatching day; Md_O is maximal diameter at oviposition day; **A=** 0.186 ± 0.052 (**t=** 3.57, **d.f.=** 120, P < $5*10^{-4}$) and **B=** 0.846 ± 0.059 (**t=**14.36, **d.f.=** 120, P < 0.0001).

observed changes of Md co-occur with the development of the longitudinal depth (a structure described by Barata 1981). These changes can be explained by the elasticity that the geometry of exochorion cells confers to this area of the egg, where cells are mainly hexagonal (Barata 1998; Chaves & Añez 2003). This pattern provides a topology which can have many different arrays in contrast with the geometry of caudal and opercular regions, where pentagonal cells are present and do not permit changes (Chaves & Añez 2003).

As seen from the linear regression, the relationship between maximal diameter at the initial (O) measurements and those at BHD is nearly constant. Differences between Md at O and BHD are principally due to the intercept value of the linear regression function. Based on this later result we postulate that the intercept could represent a constant value that reflects the maximal elasticity of an egg during its development. Notwithstanding, further embryological studies are required.

Finally, our results must be compared versus wild triatomines, as laboratory-raised bugs are shorter than wild ones (Zeledón 1981; Dujardin et al. 1999).

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