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MORPHOMETRIC DIFFERENTIATION BETWEEN TWO GEOGRAPHIC POPULATIONS OF *TRIATOMA INFESTANS* IN URUGUAY

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ABSTRACT: Metric characters have never been applied in quantitative studies on *Triatoma infestans*, the main vector of Chagas disease in the seven southernmost countries of Latin America. A first study was carried out in Uruguay, clearly differentiating two geographical populations of *T. infestans*, one from the northern Department of Rivera, the other from the southern Department of Soriano. Metric differences were also shown between administrative sections within each Department, more so in the southern region, and did not disappear after one generation in two F1 offspring, even when these were removed from their natural environment.

KEY WORDS: *Triatoma infestans*, morphometry, Chagas disease, Uruguay.

INTRODUCTION

Morphometry has been poorly exploited in Triatominae. So far, only two quantitative studies have been published for distinguishing closely related species in the *Triatoma sordida* group (GORLA *et al.*, 1993) and in the *Rhodnius prolixus* group (HURTADO GUERRERO, 1992; HARRY *et al.*, 1994), and there is no study comparing geographic populations of *Triatoma infestans* (Klug, 1834). This important vector of Chagas disease is thought to be morphologically stable across the wide territory it occupies, i.e. the seven southernmost countries of Latin America. Throughout most of its current distribution *T. infestans* seems to occupy only domestic and peridomestic habitats, living in the cracks and crevices of rural dwellings and animal enclosures. In Uruguay, two epidemiologically distinct populations have been involved on the basis of their infection rate (SALVATELLA, 1986) and the reinfestation frequency after insecticide spraying (SALVATELLA, comm. pers.). The first of these populations occupies the northern area of Uruguay (Rivera), while the other is found in the South (Soriano). These populations have been compared cytogenetically (PANZERA *et al.*, 1992). Geographical or ecological populations of *T. infestans* have been compared in Bolivia (DUJARDIN *et al.*, 1987) and in Chile (FRIAS & KATTAN, 1989) by using isozymes, revealing strong spatial structuring among subpopulations in this species (DUJARDIN *et al.*, 1994). However, since most of the changes concerned in microevolution, such as subspecific differentiation, are changes of metric characters (FALCONER, 1981), it is of interest to examine possible morphometric differences between populations.

MATERIAL AND METHODS

The insects: A total of 67 male *T. infestans* from Rivera and 147 from Soriano were captured by field workers of the National Chagas Program. Nymphs from both Departments were used to set up laboratory colonies for the study of F1 male and female offspring.

Geographic areas: Rivera and Soriano Departments differ in annual mean temperature (18,30° C in Rivera and 17,33° C in Soriano). They are subdivided into smaller administrative sections: 8 in Rivera and 13 in Soriano (Fig. 1). The origin of specimens according to these sections is indicated in Table 1.

Measurements: A monocular Olympus® SZM3 was used. Each living specimen was fixed on a mobile plate fixed to a Vernier scale, where the reading was made in millimeters. For each specimen, eight measurements were performed (Fig. 2): A (outer distance between eyes), B (inner distance between eyes), C (inner distance between ocelli), D (anteocular length), E (postocular length, including collar), F (head length, including collar), G (width of the collar), H (width at the intersection of the fore and median lobes of the pronotum), I (width of the thorax) and J (length of the thorax). All measurements were performed by the same investigator. The following ratios between measurements were also retained for the analysis: D/F, A/G, A/H, C/J, D/J, F/H, G/J, H/J and I/J. They are named: DF, AG, AH, CJ, DJ, FH, GJ, HJ and IJ, respectively.

Numerical analysis: Most of the statistics were performed using the STATA package (Computing Resource Center, 1992, Stata Reference Manual: Release 3, 5th ed., Santa Monica, CA, USA). Means, standard errors and coefficients of variation are presented in Tables 2 and 3. Comparisons between specimens were tested assuming a normal distribution and equal variances for each measure. First, a two-way ANOVA was performed revealing the contribution of Departments and sections to the significant differences found at various characters (Table 4). Next, a one-way ANOVA was performed according to each geographic subdivision, on natural populations as well as on their offspring (Table 5). An *a posteriori* test of pairwise comparisons of means was then applied according to SCHEFFE (1953), revealing more differences between sections from different Departments than within the same Department (Table 6). Finally, a stepwise maximum likelihood logistic regression model was constructed, which allowed a check on the validity of individual measurements as a diagnostic of geographical origin (Table 7).

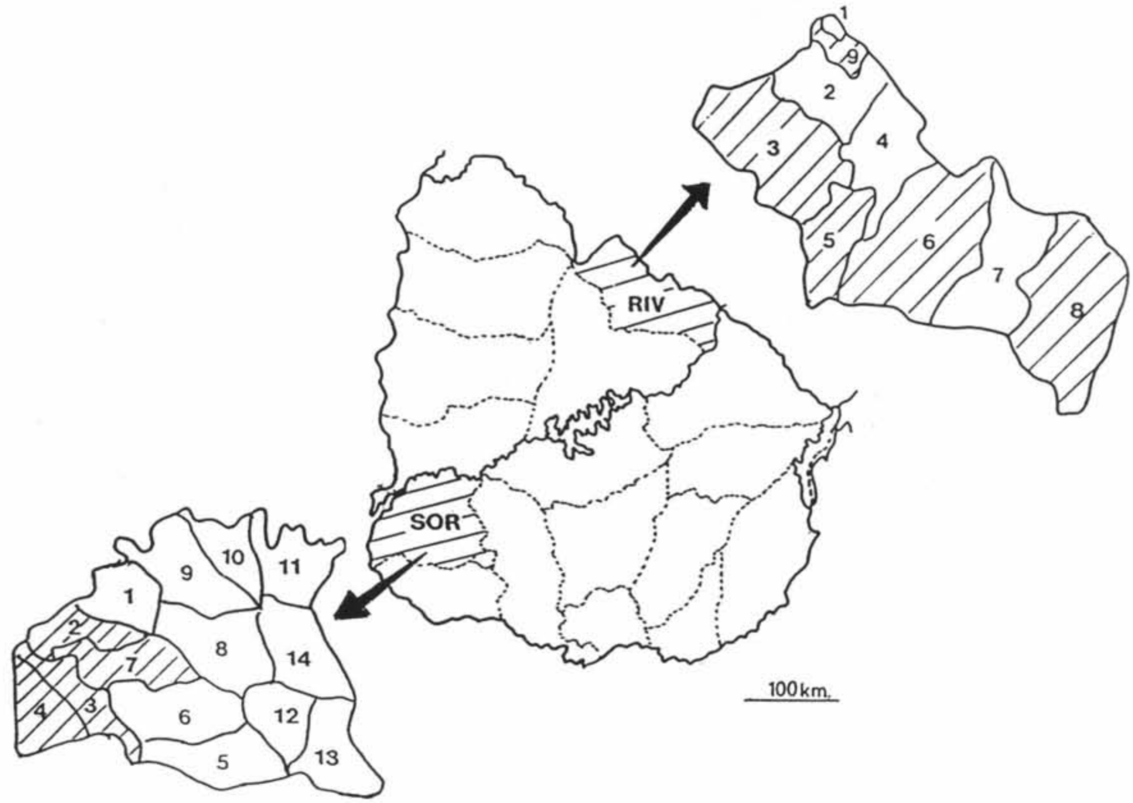

Fig. 1.– Map of Uruguay, with the two areas prospected: RIV=Rivera and SOR=Soriano (dashed zones). A magnification of both Departments shows their subdivision into administrative sections. Dashed zones are the sections the insects come from. The 100 km scales refers to the country of Uruguay.

Department	RIVERA					
Administrative sections	R3	R5	R6	R8	R9	Total 5
Number of male insects	22	5	6	9	25	Total 67
Department	SORIANO					
Administrative sections	S2	S3	S4	S7		Total 4
Number of male insects	72	43	30	2		Total 147

Table 1.– Geographic origin and number of insects: insects were collected in five administrative sections of Rivera (R3, R5, R6, R8 and R9), and in four sections in Soriano (S2, S3, S4, S7); a total of 214 insects was submitted to morphological measurements, 67 originating from Rivera, 147 from Soriano.

RESULTS

The size of male insects, in natural populations as well as in laboratory offspring, appears to be larger in Rivera than in Soriano (Table 2): the confidence interval of the mean did not overlap between Rivera and Soriano for characters A, F, I and J. The coefficient of variation, which is independent of the size of the insect, was higher for the head measures in Rivera, though it was lower for the thorax, especially for the width of thorax (I). The overall finding that means were not the same among Departments arose mainly from the contrast between some administrative sections, either between or within Departments (Table 4). Indeed, the *a posteriori* pairwise comparisons both of absolute and relative measures (Table 5) revealed 33 significant metric differences between sections (Table 6). Out of these 33 differences, 22 (66%) could be attributed to differences between sections belonging to different Departments, especially sections S2, S3 or S4 in Soriano versus section R8 or R9 in Rivera (Table 6). Further structuring was evidenced by comparing sections within Departments. Whereas just one significant difference was found between sections of Rivera (section R3 and R8 differed for the ratio A/G), there were several differences between sections within Soriano (sections S2 and S3, or S2 and S4 differ at three absolute and eight relative measures) (Table 6). The significant differences we found at A, F and J between 67 and 147 male specimens were found again comparing 13 and 5 male offspring from the corresponding localities. Females of both areas were not compared, except in the offspring population (results not shown): the 6 and 5 female offspring from different areas did not exhibit any significant difference, except for H. A correct geographic attribution (Rivera versus Soriano) of a unique individual needed a logistic regression model constructed from a set of various absolute measures, namely A, D, E, F, H and J. Out of the 172 specimens that this model at-

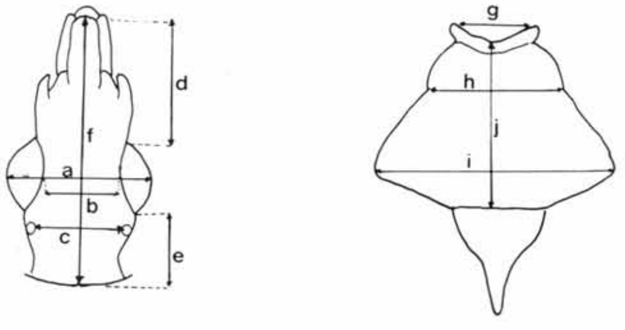

Fig. 2.– Measurements performed on the head (left hand of the figure): a=outer distance between eyes; b = inner distance between eyes; c=inner distance between ocellae; d = anteocular distance; e=postocular distance (including neck); f=head length (including neck). Measurements performed on the thorax (right hand of the figure): g=width of the collar; h=width at the intersection of the fore and median lobes; i=width between humerus; j=length of the thorax (excluding the scutellum). Symbols a, b, c, ..., j are referred to in uppercase in the text (A, B, C, ..., J).

	Rivera (67)			Offspring (5)		
	Means	SE	CV	Means	SE	CV
A	2,373	0,013	4,556	2,500	0,035	3,162
B	1,037	0,009	7,154	1,110	0,019	3,769
C	1,258	0,009	5,935	1,310	0,019	3,193
D	2,429	0,015	5,170	2,440	0,029	2,672
E	1,220	0,014	9,625	1,310	0,043	7,342
F	4,702	0,022	3,789	4,990	0,064	2,869
G	2,255	0,012	4,317	2,360	0,029	2,762
H	3,249	0,018	4,523	3,390	0,019	1,234
I	5,771	0,019	2,765	6,000	0,035	1,318
	Soriano (147)			Offspring (13)		
	Means	SE	CV	Means	SE	CV
A	2,310	0,008	4,035	2,354	0,022	3,298
B	1,024	0,006	6,771	1,008	0,015	5,300
C	1,253	0,005	5,129	1,227	0,011	3,163
D	2,404	0,010	4,918	2,481	0,022	3,244
E	1,202	0,009	9,097	1,269	0,036	10,240
F	4,591	0,014	3,740	4,758	0,050	3,810
G	2,232	0,008	4,176	2,269	0,022	3,546
H	3,225	0,011	4,269	3,185	0,027	3,033
I	5,682	0,020	4,181	5,739	0,039	2,468
J	3,869	0,018	5,672	3,915	0,048	4,436

Table 2.– Means, standard errors and coefficients of variation of morphological characters. SE=standard error, CV=coefficient of variation (the ratio between the standard error and the mean, multiplied by 100). Letters refer to the different parts of the head (A to F) or the thorax (H to J): A=outer distance between eyes; B=inner distance between eyes; C=inner distance between ocelli; D=anteocular distance; E=postocular distance (including collar); F=head length (including collar); G=width of the collar; H=width at the intersection of the fore and median lobes; I=width between humerus; J=length of the thorax (excluding the scutellum). Between brackets, the number of specimens.

	Rivera (67)				Offspring (5)		
	Means	SE	CV		Means	SE	CV
DF	0,517	0,002	3,698	DF	0,489	0,010	4,635
AG	1,054	0,006	5,043	AG	1,060	0,021	4,497
AH	0,731	0,004	4,844	AH	0,738	0,011	3,201
CJ	0,316	0,003	7,207	CJ	0,325	0,004	3,071
DJ	0,610	0,005	7,053	DJ	0,605	0,012	4,271
FH	1,450	0,009	5,043	FH	1,472	0,018	2,799
GJ	0,566	0,004	5,278	GJ	0,585	0,009	3,332
HJ	0,815	0,005	4,618	HJ	0,841	0,008	2,255
IJ	1,448	0,008	4,350	IJ	1,488	0,011	1,604
	Soriano (147)				Offspring (13)		
	Means	SE	CV		Means	SE	CV
DF	0,524	0,002	3,956	DF	0,522	0,003	2,345
AG	1,036	0,003	4,096	AG	1,039	0,015	5,071
AH	0,717	0,002	3,970	AH	0,739	0,006	2,971
CJ	0,325	0,002	6,439	CJ	0,314	0,003	3,120
DJ	0,623	0,003	6,100	DJ	0,634	0,007	3,933
FH	1,425	0,004	3,619	FH	1,495	0,015	3,653
GJ	0,578	0,003	5,408	GJ	0,581	0,009	5,529
HJ	0,835	0,003	4,573	HJ	0,814	0,007	3,220
IJ	1,471	0,005	4,466	IJ	1,468	0,017	4,126

Table 3.– Means, standard error and coefficient of variation of morphological ratios. Abbreviations as for Table 2.

	Absolute measures								
Metric	A	C	D	E	F	G	H	I	J
Model	0,000	0,037	0,000	0,000	0,000	0,018	0,006	0,008	0,000
Dep	0,108	0,821	0,757	0,047	0,213	0,092	0,202	0,934	0,066
Sec	0,007	0,024	0,000	0,000	0,024q	0,028	0,005	0,066	0,000
	Relative measures								
Metric	DF	AG	AH	CJ	DJ	FH	GJ	HJ	IJ
Model	0,000	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000
Dep	0,113	0,232	0,748	0,208	0,199	0,011	0,135	0,387	0,027
Sec	0,000	0,000	0,028	0,000	0,000	0,000	0,000	0,001	0,000

Table 4.– Two-way ANOVA: results showed as the probability of finding a value of F larger than the calculated value. Model=probability for the total analysis. Dep=probability for the first factor, i.e. the Rivera and Soriano Departments. Sec=probability for the second factor, i.e. the administrative sections of Rivera and of Soriano. This analysis was performed on the absolute (top) and relative (bottom) measures separately.

tributed to Soriano, 134 (78%) were correctly classified, while this predictive value was lower (69%) for the specimens attributed to Rivera (Table 7, top). The same model applied to the offspring allowed a correct classification of 72% (13/18) of the offspring (Table 7, bottom).

	Natural populations			Offspring		
Metric	F	Pr>F	Bartlett	F	Pr>F	Bartlett
A	19,1	0,000	–	12,7	0,003	–
B	1,49	0,223	–	14,7	0,002	–
C	0,23	0,630	–	15,9	0,001	–
D	2,04	0,155	–	1,01	0,329	–
E	1,16	0,283	–	0,40	0,536	–
F	18,6	0,000	–	6,55	0,021	–
G	2,70	0,102	–	5,03	0,039	–
H	1,30	0,255	–	20,5	0,003	–
I	7,84	0,006	+	14,9	0,001	–
J	15,8	0,000	–	2,07	0,170	–
DF	5,52	0,019	–	15,6	0,001	–
AG	6,91	0,009	+	0,63	0,440	–
AH	10,1	0,002	+	0,02	0,877	–
CJ	8,03	0,005	–	4,64	0,047	–
DJ	4,87	0,028	–	4,82	0,044	–
FH	8,00	0,005	+	0,69	0,420	–
GJ	7,55	0,006	–	0,09	0,769	–
HJ	13,2	0,000	–	4,19	0,058	–
IJ	5,90	0,006	–	0,50	0,439	–

Table 5.– One-way ANOVA: this analysis compares the means of both Departments, Rivera and Soriano. Natural populations of Rivera (67 individuals) and of Soriano (147 individuals) are tested on the left of the table, and corresponding male offspring on the right (5 individuals from Rivera and 13 from Soriano). F=calculated value; Pr>F=probability of finding a larger value; Bartlett=it tests the hypothesis of equal variances: - = non significant Bartlett's test; + =significant Bartlett's test (unequal variances). Underlined values of Pr>F are significant results with a negative Bartlett's test.

	S2	S3	S4	S7	R3	R5	R6	R8	R9
	(72)	(43)	(30)	(2)	(22)	(5)	(6)	(9)	(25)
S2	/								
S3	6	/							
S4	4	n	/						
S7	n	n	n	/					
R3	2	n	n	n	/				
R5	n	n	n	n	n	/			
R6	n	n	n	n	n	n	/		
R8	7	3	3	n	1	n	n	/	
R9	4	3	n	n	n	n	n	n	/

Table 6.– Number of significant (P<0,02) metric differences, at both relative and absolute measures, as estimated by the Scheffé test after one-way ANOVA. S=Soriano; R=Rivera; S2, etc.=administrative section number 2, etc. of Soriano; R3, etc.=administrative section number 3, etc. of Rivera; n=no significant difference. The metrics at which differences were found are partly detailed in this legend. For example, between S2 and S3 (in the same Department) there are seven significant differences (two absolute measures, E and J, and four relative ones, CJ, DJ, GJ, HJ and IJ), between S2 and S4 there are four significant differences (one absolute measures, D, and three relative ones, DF, CJ and DJ), and between sections R3 and R8 of Rivera there is only one difference (the ratio AG). Most of the differences (22 out of 33) are found, however, between sections belonging to different Departments. Between brackets, number of specimens.

Natural populations			
Observed	*Classified*		
	Soriano	Rivera	
Soriano	134	13	147
Rivera	38	29	67
Total	172	42	214
Offspring			
Observed	*Classified*		
	Soriano	Rivera	
Soriano	9	4	13
Rivera	1	4	5
Total	10	8	18

Table 7.– Diagnostic of geographic origin. A stepwise procedure allowed us to select from among the absolute measures of the following set: A, D, E, F, G, H and J, as the most interesting combination of metrics for diagnosing of geographic origin (maximum likelihood logistic regression). Ratios had a lower predictive power. In natural populations, our logistic regression model correctly attributed to Soriano 78% (134/172) of the specimens it classified there, whereas the predictive value for Rivera fell to 69% (29/42). It correctly classified 91% (134/147) of the Soriano natural specimens, but no more than 43% (29/67) of the Rivera specimens. Nevertheless, a satisfactory classification in the offspring was possible: out of the 13 specimens whose parents originated from Soriano, 9 (69%) were recognised by the model, while 4 out of 5 (80%) were correctly classified to their Rivera origin.

DISCUSSION

Geographic origin of the populations

Rivera and Soriano Departments differ in annual mean temperature (18,30° C in Rivera and 17,33° C in Soriano). They also represent two socio-economically distinct areas, offering different conditions for the vector micro environment. Dwellings in Rivera are made of wood or adobe, while brick and cement are the main construction materials used in Soriano. These characteristics of the habitat of *T. infestans*, as well as its infection rate by the parasite *Trypanosoma cruzi*, heavier in Rivera than in Soriano (SALVATELLA, 1986), or temperature differences, could be amongst the factors affecting the morphometry of both populations. Historical data suggest a two-way entry of *T. infestans* into Uruguay, northern populations coming from Brazil during the 19th century and southern populations coming from Argentina hundreds of years before (SALVATELLA, 1991). Metric differences between specimens from the North and South of Uruguay also lend support to the hypothesis of a different geographic origin of these populations.

Genetic basis of the metric characters

Our results, which included (1) significant metric differences between Soriano and Rivera, and (2) more differences within Soriano than Rivera, were in agreement with previous cytogenetical findings revealing differences between Rivera and Soriano, and more genetic structuring within Soriano (PANZERA *et al.*, 1992). This genetic variation could account for the metric differences; however, metric characters are under environmental control as well, and the contribution of each parameter, genetic background and environment, should be clarified. The study of two F1 crosses, one from a Rivera and the other from a Soriano couple, allowed two first observations to be made: 1) the persistence in a new environment of the same morphometrical differences as those observed between natural populations; 2) the appearance of other significant differences: among these, offspring B, C, G and H were also significantly different (Table 3). The first observation indicates that environmental influence acts slowly on the metric characters used here. Indeed, the differences between Soriano and Rivera did not disappear in an artificial, common environment, after more than one generation. The second observation could reflect a differential sensitivity to environment according to metric character. Interestingly, half of the differences of ratios between natural populations disappeared in the insectary (Table 5).

Biological relevance

Using monofactorial characters such as isoenzyme variability or heterochromatin block distribution, no sibling species have been detected in *T. infestans*, but local geographical differentiation has been demonstrated (DUJARDIN *et al.*, 1987, 1994; PANZERA *et al.*, 1992). Metric characters give these studies additional arguments lending support to the hypothesis of differentiated local populations within the wide geographical extension of this species.

Epidemiological relevance

Control of *T. infestans* currently relies on spraying infested houses with residual insecticides, followed by long-term surveillance to detect reinfestations. But it is generally difficult to determine the source of reinfestations - whether survivors from the initial treatment, or immigrants from untreated foci (DIAS, 1987). The immediate pay-off of this study is the hope that morphometry could bring a simple and low-cost tool for monitoring the control programmes. The persistent metric differences between sections from Rivera (North of Uruguay) and from Soriano (South of Uruguay) argue against the idea that reinfestations occurring sporadically in one area are the result of reinvasion from the other one, and reinforce the hypothesis that reinfestation would result from recrudescence of residual populations, not eliminated by insecticide spraying. Morphometric analysis during the surveillance phase of a control programme may therefore be helpful in understanding reinfestation, and, as a consequence, in making decisions during the activities.

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