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SHORT COMMUNICATION

The origin of *Rhodnius prolixus* in Central America

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Rhodnius prolixus Stal (Hemiptera, Reduviidae) is the main domestic vector of Chagas disease in Venezuela and Colombia, and also in parts of Central America (El Salvador, Guatemala, Honduras and Nicaragua). In Central America it seems to be confined entirely to domestic and peridomestic habitats, whereas silvatic populations have been recognized in various parts of Venezuela and Colombia, mainly in palm tree crowns, which are assumed to represent the original ecotope of this species (e.g. Gamboa, 1963).

The distribution of *R. prolixus* shows a clear discontinuity between South and Central America, since it has never been recorded from Panama nor from southern Costa Rica. The most widely cited explanation of this discontinuity invoked the passive carriage of eggs and young nymphs in the plumage of storks, *Mycteria americana*, which often nest in palm tree crowns and are known to migrate between the two regions (Gamboa, 1962, 1963). However, this does not explain why silvatic populations are not found in Central America, nor in other regions where *M. americana* can be found, such as the humid chaco of Paraguay. An alternative hypothesis based on historical records (Zeledón, 1996) suggests that *R. prolixus* in Central America was derived from an accidental escape of laboratory-bred insects in 1915, which were subsequently transported in association with people visiting different rural areas (Schofield & Dujardin, 1997). In this way, *R. prolixus* in Central America would have invaded several countries over a few decades as a domestic species*, in much the same way that the related *Triatoma infestans* (Klug) colonized the southern cone countries during the last 100 years (Schofield, 1988).

The question is of more than academic interest because if, indeed, the Central American populations are of recent origin from a small source population, then they would represent a much more amenable target for the recently announced Chagas disease vector control initiative in Central America (WHO, 1997). The hypothesis implies that, due to a founder effect, *R. prolixus* in Central America would show less genetic variability relative to conspecific populations in South America. Moreover, paralleling the morphometric changes seen in *T. infestans* from different points along its supposed migrations, we expected that Central American *R. prolixus* would show reduced size variables compared to its South American populations. To test these ideas, we compared Colombian and Honduran populations of *R. prolixus* by multi-locus isoenzyme electrophoresis, RAPD analysis, and morphometry.

Thirteen Honduran specimens (twelve males, one female) and eight Colombian specimens (all males) of *R. prolixus* were collected from infested houses. The insects were dissected for isoenzyme analysis of thoracic muscles and RAPD analysis of genomic DNA isolated from the legs. The heads and wings were retained for morphometric measurements. Cellulose acetate electrophoresis for 12 enzyme systems followed Richardson *et al.* (1986) (cf. Dujardin *et al.*, 1997a). For the twelve enzyme systems a total of seventeen loci could be consistently scored, of which none showed any variation between the two populations. DNA extraction and analysis followed the procedure of Carlier *et al.* (1996). A single 10-nucleotide oligomer of random sequence containing at least 50% G-C served as primer for each reaction, and we screened four primers A and two primers B (Operon Technologies Inc.). We scored only those bands that were well amplified and showed clear presence or absence of polymorphisms. They showed consistent differences between

*In Central America, *R. prolixus* was first recorded from El Salvador by Neiva (1915). By 1934 it had reached neighbouring Guatemala (de León, 1943) and spread into Honduras and Nicaragua during the 1950s (Dias, 1952; León-Gómez, 1963). It was found in the Guanacaste province of northern Costa Rica in 1953, apparently brought by travellers from Nicaragua (Ruiz, 1953) but appears to have been subsequently eliminated from Costa Rica by insecticide spraying. In contrast, *R. prolixus* has been known from Venezuela and Colombia since its original description by Stal (1859), and Gamboa (1962) cites a 1571 chronicle which apparently describes the insects and their habits in coastal Venezuelan settlements.

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Table 1. RAPD bands for *R. prolixus* from Honduras and Colombia.

	Total bands		Unique bands		Shared bands	Hedrick's probabilities		
Primer	Colombia	Honduras	Colombia	Honduras	Col. + Hon.	*Uch*	*Uhc*	*G*
A3	10	6	4	0	6	0.30	0	0.57
A4	8	3	5	0	3	0.33	0	0.68
A7	10	3	7	0	3	0.18	0	0.88
A18	6	5	3	2	3	0.28	0.13	0.73
B6	10	4	6	0	4	0.24	0	0.80
B8	6	8	3	5	3	0.18	0.11	0.61
Total	50	29	28	7	22			
Mean*	8.3	4.8	4.7	1.2	3.7	0.25	0.04	0.71

* Means for Honduran and Colombian specimens are significantly different both for total bands and unique bands (Kruskal–Wallis test, $P = 0.02$).

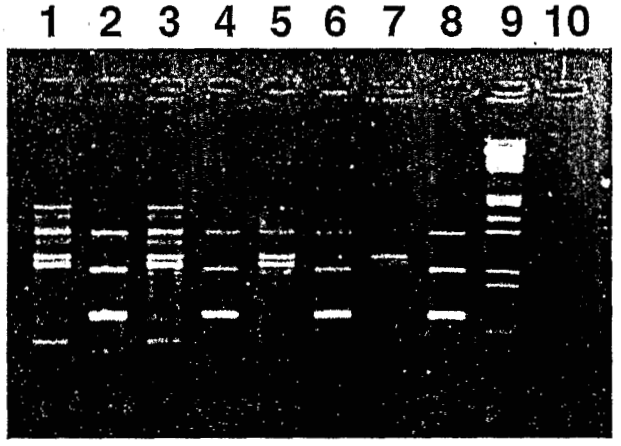

Fig. 1. RAPD banding patterns of *Rhodnius prolixus* from Honduras (lanes 1, 3, 5, 7 and 9) and from Colombia (lanes 2, 4, 6 and 8) using primer OPA-4 (Operon Technologies Inc.), except lane 9 which used primer lambda/HindIII+EcoR1 (Promega).

the two populations (Table 1; Fig. 1). The mean number of bands by primer for Colombian specimens (8.3) as well as the average number of unique bands (4.7) were significantly higher compared to Honduran specimens (4.8 and 1.2, respectively). Colombian specimens shared only 44% of bands with Honduran specimens (22/50) whereas Honduran specimens shared 76% of bands with Colombian specimens (22/29). According to statistics using genotype, rather than gene frequencies, the 'probability of unique genotype' (*U*) (Hedrick, 1975) in Colombia, compared to Honduras (*Uch* = 0.25) was much higher than in Honduras compared to Colombia (*Uhc* = 0.04), while Hedrick's 'probability of genotypic identity' (*G*) between the two countries (Hedrick, 1971) was 0.71.

Univariate and multivariate analyses were performed on the seven head and three wing measurements of each male specimen, as already described for *T. infestans* (Dujardin *et al.*, 1997b,c). A nonparametric test (Kruskal & Wallis, 1952) for the null hypothesis of identical population medians, rather than means, revealed that all measures were significantly smaller for the Honduran specimens, except for the synthlipis and external diameter of the eyes. Principal components were computed from the covariance matrix of log-transformed measures, except for the total head length, of which the first principal component is considered as a general size variable (cf. Bookstein, 1989). A plot of the first two principal components graphically illustrates the metric differences between Honduran and Colombian specimens (Fig. 2a).

Although it is well established that *R. prolixus* shows little enzyme variation (e.g. Dujardin *et al.*, 1991; Harry *et al.*, 1992; Solano *et al.*, 1997) the complete lack of isoenzyme polymorphism in both our populations could be attributed to small sample size. But the isoenzymatic identity confirms the species homogeneity of our samples and supports the idea of a recent separation between South and Central American populations – too recent to be revealed by enzyme gene mutations. By RAPD analysis, the significantly fewer bands presented by Honduran specimens (Fig. 1) fits the idea of this being the more recently colonized region and, under similar assumptions of gene neutrality, Hedrick's probabilities of unique genotype would be significantly higher in the source population (Hedrick, 1975) which was indeed the case for the Colombian specimens. An analogous argument for considering that the Honduran specimens represent a derivative population is the consistently smaller dimensions of these insects. This reduction in size (Fig. 2a) parallels the trend seen in *T. infestans* from their origin in the Bolivian Andes to the more peripheral areas of recent colonization (Fig. 2b).

The evidence presented here supports the idea that *R. prolixus* in Central America represents a genetically limited subset derived recently from the original South American populations. The parallel with *T. infestans*, spread from its Bolivian origin in association with human migrations and now being eliminated by the Southern Cone Initiative against Chagas disease (cf. Schmunis *et al.*, 1996; WHO, 1997), encourages planning for the elimination of *R. prolixus* (Schofield & Dujardin, 1997) as a primary objective of the Central American Initiative against Chagas disease.

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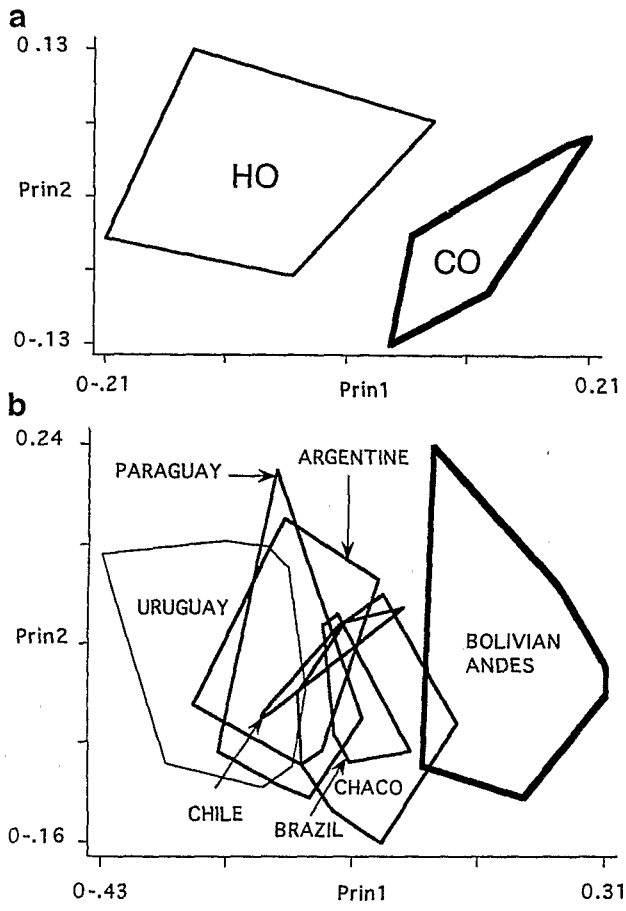

Fig. 2. First and second principal component plots for head morphometry (a) the twelve males of *R. prolixus* from Honduras (HO) and eight from Colombia (CO) projected onto the first (Prin1, 67%) and second (Prin2, 14%) principal components. The hulls enclose all specimens from each geographical area. The position of the Honduran specimens along the first principal component indicates a significantly smaller overall size compared to the Colombian specimens ($P = 0.0013$). (b) similar analysis for *T. infestans*, involving 176 adult males from natural populations in Argentina, Bolivia (Andes and Chaco regions), Brazil, Chile, Paraguay and Uruguay. The analysis indicates a cline in overall size from the supposed origin of this species in the Andean region of Bolivia to the more recent areas of colonization such as Uruguay.

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