Microdistribution of Sylvatic Triatomine Populations in Central-Coastal Ecuador

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ABSTRACT Chagas disease is a serious public health problem in Ecuador, where nearly 230,000 individuals show *Trypanosoma cruzi* infection. Sylvatic *T. cruzi* transmission is a threat to current control strategies. This is because of the possibility of house reinfestation by sylvatic triatomines after insecticide treatment. This work quantified the spatial distribution of triatomines in sylvatic habitats and its relationship with nearby human dwellings. A simple random sampling design using live-baited traps and manual searches for triatomines was used in areas near human communities in Manabí province, Ecuador, during June and July 2007. We identified risk factors associated with triatomine density using generalized linear models, and developed predictive maps for triatomine density interpolation. There were 345 triatomines belonging to the species *Rhodnius ecuadoriensis* and *Panstrongylus howardi* collected in sylvatic areas. Spatial analyses revealed an aggregated distribution pattern of the sylvatic triatomine populations (clustered mostly at a distance smaller than 100 m). Generalized linear models showed that the distance from the nearest house, nest type, and height from ground level were the main factors explaining triatomine densities. Squirrel nests (*Sciurus stramineus*), located in plants other than palms, above 5 m and close to the domicile presented higher infestation. Interpolation maps of triatomine microdistribution are presented as potential tools to predict triatomine occurrence. The presence of sylvatic populations and the synanthropic tendency of the vectors highlight the need for continuous active and passive entomological surveillance for the long-term control of Chagas disease.

KEY WORDS Chagas disease, random sampling, sylvatic habitat, triatomine ecology, Ecuador

In Ecuador, around 3.8 million people are at risk of acquiring Chagas disease (American trypanosomiasis) and >230,000 individuals are currently infected with the disease making it a serious public health issue (World Health Organization [WHO] 2002). Sixteen species of insect vectors (Hemiptera: Triatominae) have been reported in Ecuador (Abad-Franch et al. 2001; Lent and Wygodzinsky 1979). *Triatoma dimidiata,* thought to be an introduced species, is considered the main vector, and *Rhodnius ecuadoriensis* is considered the second most important vector in the country. *T. dimidiata* seems to be exclusively domestic in Ecuador, but *R. ecuadoriensis* presents domiciliary and peridomiciliary infestations, and abundant sylvatic populations in the central coastal region where it is associated with the endemic palm species *Phytelephas aequatorialis* (Abad-Franch et al. 2001, Grijalva et al. 2005). Several other triatomine species have also shown capacity to colonize houses and to transmit *Trypanosoma cruzi,* including *Panstrongylus howardi* in dry areas of Manabí province, and *P. rufotuberculatus* in the Coastal region and Interandean valleys. In the Amazon region of Ecuador, the sylvatic vectors *R. pictipes, R. robustus,* and *P. geniculatus* are responsible for most of the *T. cruzi* infections, transmitting the parasite without establishing colonies in human environments (Aguilar et al. 1999), but little information exists regarding the ecology of most triatomine species in Ecuador.

The main objectives of the current study were to develop a better-standardized method for sampling sylvatic triatomine populations and to evaluate the efficacy of live-baited traps compared with manual searches. We also sought to identify environmental factors that may influence the distribution pattern of these sylvatic populations, and to model the spatial distribution of sylvatic triatomine populations in relation to their location near human dwellings.

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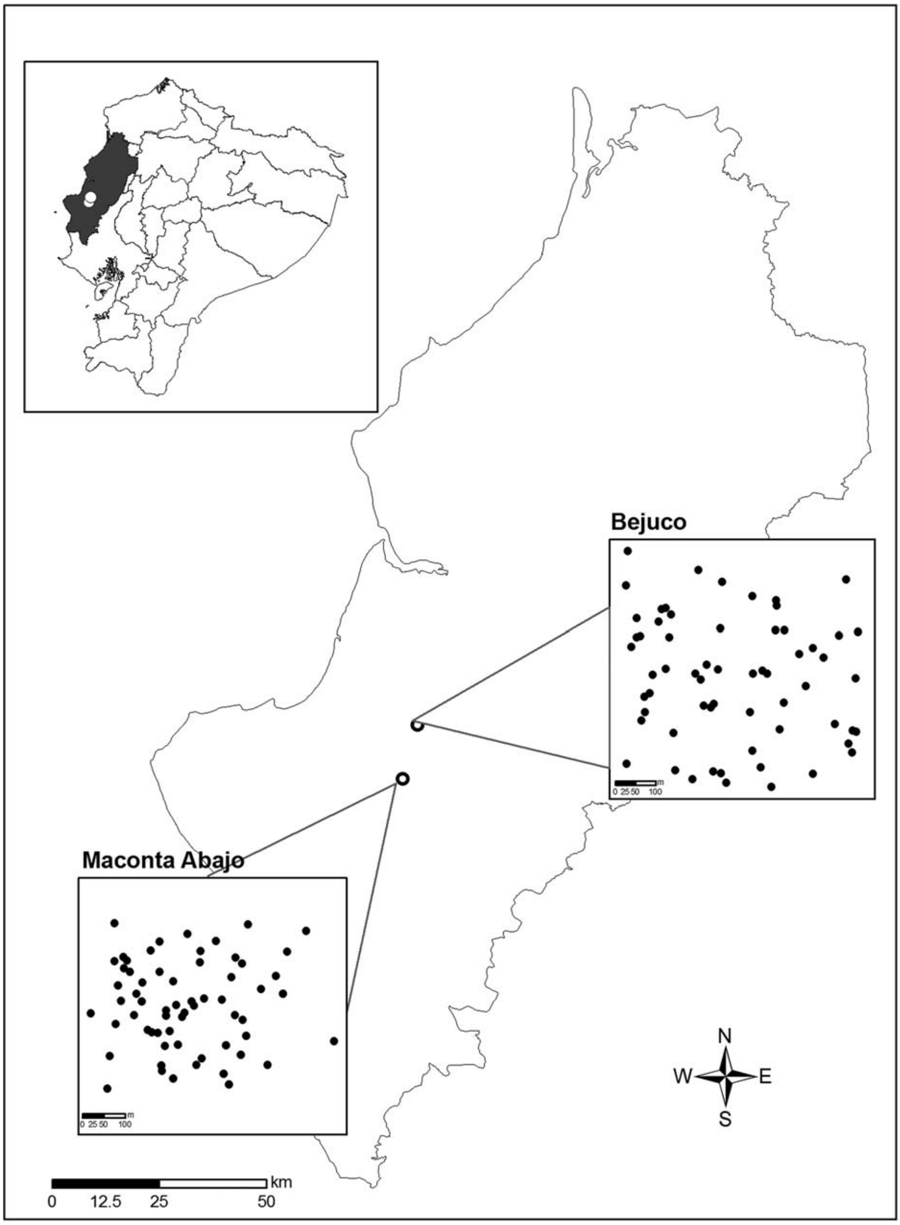

Fig. 1. Map of Manabí province showing the location of the studied communities and their respective random point patterns for triatomine sampling.

Materials and Methods

Study Area. Triatomine bugs were collected during June and July, 2007, in two rural communities in Portoviejo County, Manabí province [El Bejuco (80°19′34.12″W; 0°57′24.75″S; 248 m.a.s.l.) and Maconta Abajo (80°20′53.52″W; 1°5′40.88″S; 290 m.a.s.l.)], located in central coastal Ecuador (Fig. 1). These communities were chosen for the high triatomine household infestation rates they showed in previous visits (12.5% El Bejuco and 18.42% Maconta Abajo) and the presence of nearby forested areas. Both communities had been surveyed for triatomine infestation 6 mo earlier and houses found to be infested had been sprayed with deltamethrin at 25 mg a.i./m^2. El Bejuco community has 98 houses and around 450 inhabitants, and Maconta Abajo has 51 houses and around 110 inhabitants. During the collection period, the average temperature in the communities was 23.0 ± 3°C and the average relative humidity ranged between 90 and 97%. Temperature and humidity data were obtained using dataloggers (LogTag Recorders-Trix-8, Hong Kong, China). The two parameters were measured every 30 min for 24 h in 10 sampling points within each quadrat.

The vegetation in the study areas represents a semideciduous forest of the Ecuadorian lowlands (Cerón et al. 1999) characterized by thorny vegetation, a disperse tree stratum and dense understory with plants that lose their leaves once a year (examples of characteristic flora are *Ceiba* spp., *Eriotheca ruizii*, *Triplaris cumingiana*, and *Prosopis juliflora*). Both study areas had a mix of forest, and cultivated patches, primarily with corn and papaya crops.

Triatomine Sampling Design. In each community, a quadrat (600 × 600 m) was defined in a sylvatic area near houses of the village (100–200 m from a domicile) that provided places for sampling. Within each quadrat, 62-point coordinates were randomly generated using R Software (R Development Core Team 2007, Spatstat package, Fig. 1). These coordinates were then transferred to Geographical Positioning System receivers (GPS, Meridian Platinum, Magellan, San Dimas, CA) for field sampling. At each point, manual searches for triatomines were performed for 30 min by three-person teams in different microhabitats (nests, burrows, tree holes, and under trunks and rocks) within a 10 m radius area. In each microhabitat, information regarding vertebrate fauna, vegetation type, and nest height from ground level was recorded. Additionally, 45 live-baited traps (Noireau et al. 2002) were placed at 15 points in each quadrat, selected for having more availability of microhabitats; traps were placed for a 24 h period. This sampling method has proved to be effective in sampling Triatominae from palm trees (Noireau et al. 2002) but had not previously been tried for other habitats in Ecuador. Both methods, live-baited trap and manual searches, were compared with determine their efficacy for triatomine collection at the 15 points where both methods were applied.

Sampling of the domiciles and surrounding peridomiciles within the studied communities was performed in parallel, by means of timed manual collections, as previously described by Grijalva et al. (2005).

Triatomines found in each microhabitat were placed in labeled plastic containers and transported live to the Insectary at the Center for Infectious Disease Research, Catholic University, Quito, Ecuador, where they were counted and identified to species. Species identification was based on morphological criteria (Lent and Wyzodzinsky 1979, Carcavallo et al. 1998) and comparison with pinned specimens deposited in the Entomology Museum of the Catholic University (QCAZ) in Quito, Ecuador.

Data Analysis

Entomological Indices. Four entomological indices were calculated: (1) infestation index (No. positive houses or nests/ No. houses or nests examined × 100), (2) density (No. triatomines collected/ No. houses or nests examined), (3) crowding (No. triatomines collected/ No. positive houses or nests), and (4) colonization index (No. houses or nests with nymphs/ No. positive houses or nests × 100) (WHO 2002, Grijalva et al. 2005). A house or nest was considered positive when living triatomines were found in the place.

Triatomine Infection. To assess triatomine infection a visual analysis of feces and intestinal content under the microscope was performed to look for *T. cruzi* alike individuals. Then a polymerase chain reaction confirmation was carried out with S35 and S36 primers.

For the distributional analyses, triatomine abundance data were homogenized by log transformation ($\log(x + 1)$) to reduce the importance of occasional large abundance values (Clarke 1993).

Point Pattern Analysis. The distribution of positive sampling points (where triatomines were found) was analyzed using the coordinates of each point and the vertex of the determined quadrat, by means of the nearest neighbor distance function $G(r)$ (Ripley 1981) in R software. This function represents the correlation of the cumulative function G of the r distance from a random point to the closest one. The distribution pattern (random, aggregated, or regular) of the points was evaluated by comparing the observed data with the null model of complete spatial randomness (CSR, Ripley 1981). We computed upper and lower 95% confidence intervals (CI) using 1000 simulations to evaluate the spatial distribution pattern.

Generalized Linear Models. Generalized linear models (GLMs) using a log link were used to test for associations between environmental factors and the number of triatomines found in each sampling point of both communities. The factors included in the model were the type of nest, the type of plant, the height from ground level, and the distance to the nearest house. Change in triatomine abundance because of each factor was modeled considering each factor independently and in combination with others. The most parsimonious model was identified using the value of Akaike's Information Criterion (AIC, Venables and Ripley 2002). These analyses were performed using the mass library for R (R Development Core Team 2007).

Relation Between Triatomine Abundance and the Distance to the Nearest House. Because GLM analyses revealed that the distance to the nearest house was the most important factor explaining triatomine abundance, we further quantified this relationship using Tablecurve 2D v. 5.01 (SYSTAT Software Inc. 2002). Based on R^2 values, a Lorentzian cumulative function was picked for the observed probability distribution. Distance values started at 100 m because triatomine sampling data in houses (distance = 0) were collected by a different method (Grijalva et al. 2005). Fit was realized by the Lorentzian cumulative function, equation:

$$y = \frac{a}{\pi}\left[\arctan\left(\frac{x - b}{c}\right) + \frac{\pi}{2}\right],$$

with values of $F = 81.52$, $R^2 = 0.958$, and the parameters transition height: a = 12.14, transition center: b = 235.32 and transition width: c = −38.85.

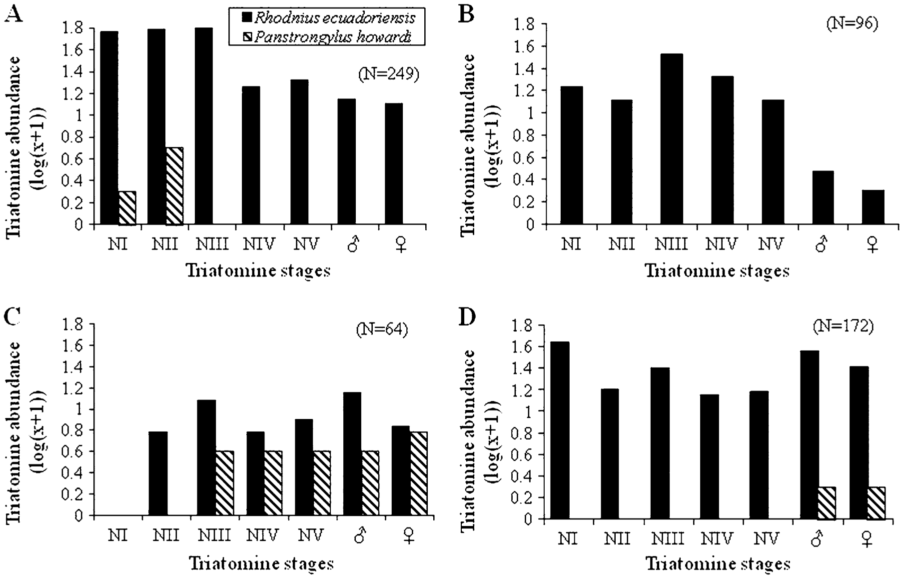

Fig. 2. Triatomine abundance frequency in sylvatic quadrats and parallel sampling in domicile and peridomicile in the two communities sampled in Manabí province. The frequency of specimens of the five nymphal stages: NI, NII, NIII, NIV, and NV, and the adults separated by sex, males (♂) and females (♀) are shown. Population structure of the species *R. ecuadoriensis* and *P. howardi* in (A) sylvatic quadrat of the El Bejuco community, (B) sylvatic quadrat of the Maconta Abajo community, (C) domicile and peridomicile of the El Bejuco community, and (D) domicile and peridomicile of the Maconta Abajo community. The total number (*n*) of specimens for each species in each community is given.

Density Interpolation. Predictive maps of triatomine densities were developed by combining information of the number of triatomines collected at the coordinates of the 62-quadrat points. The data were adjusted at a surface z(x,y) form, by means of the Gridfit function of the Software MATLAB 7.0 (D'Errico 2006). Interpolations relating information between nearest neighbors were obtained to develop a Gridfit-modeled surface compiled with the plot of the sampled points.

Results

Triatomine Sampling. A total of 345 specimens was collected within the two sampled quadrats (Fig. 2A and B). Live-baited traps collected only one specimen, a third stage nymph in a palm (*P. aequatorialis*). Ninety-nine percent of all collected specimens were *R. ecuadoriensis.* The remaining five specimens were *P. howardi* collected in El Bejuco community. Two of the *P. howardi* (NII, Fig. 2A) were collected in the peridomicile in a mouse nest (*Mus musculus*). The remaining three specimens (one NI and two NII) were found in a mouse nest as well in an *Aiphanes eggersii* palm and at a distance of 255 m from the nearest house.

Specimens of *R. ecuadoriensis* were found in bird nests (feather nests from *Campylorhynchus fasciatus* (Passeriformes: Troglodytidae) and woven stick nest from other nonidentified species) and in mammal nests: *Didelphis marsupialis* (Didelphimorphia; Didelphidae), *Mus musculus* (Rodentia: Muridae) and *Sciurus stramineus* (Rodentia: Sciuridae). In the two sampled areas, these nests had been located in 13 different plant species: *Aiphanes eggersii, Phytelephas aequatorialis, Bromelia pinguin, Jatropha curcas, Caesalpinia* sp., *Samanea saman, Senna* sp.1, *Senna* sp.2, *Casearia* sp., *Sapindus saponaria, Guazuma ulmifolia, Trema micrantha, Aegiphila* sp.

As a general pattern, we collected a high number of first nymphal stages but few adults of *R. ecuadoriensis* in sylvatic habitats, which contrasted with the higher proportion of adults collected in the peridomicile habitats (Fig. 2). No intradomicile specimens were found during this investigation. A substantial number (20 specimens) of *P. howardi* was found in peridomicile areas.

An infestation index of 12.5 and 13.5% was determined for El Bejuco and Maconta peridomiciles, respectively, and the microhabitat infestation index found in the sylvatic quadrats was 19.8 and 30%, respectively, for these communities (Table 1). Squirrel nests presented higher rates of infestation and abundance of *R. ecuadoriensis,* compared with mouse or bird nests, and overall abundance was higher in other plants compared with palm trees (Table 2).

Infections with *T. cruzi* were detected in 63% of the 16 *P. howardi* and 27% of the 56 *R. ecuadoriensis* individuals analyzed from the peridomicile, and in 32% of the 25 *R. ecuadoriensis* individuals analyzed from the sylvatic habitats.

Point Pattern Analysis. In both communities the $G(r)$ function revealed that the distribution of positive sampling points was significantly aggregated (Fig. 3; the observed data curve lies above the CSR curve, and above the 95% CI, not shown). Such an aggregation occurred in a distance between points of 70 m (Bejuco) and 100 m (Maconta Abajo).

Table 1. Entomological indices calculated for the peridomicile and sylvatic quadrat from *R. ecuadoriensis* and *P. howardi* individuals found in the two sampled communities

Community	Peridomicile				Sylvatic area			
	Infestation index (%)	Density index	Crowding index	Colonization index (%)	Infestation index (%)	Density index	Crowding index	Colonization index (%)
El Bejuco	12.5	1.3	10.7	88.9	19.8	1.3	6.6	86.8
Maconta Abajo	13.5	1.8	13.6	100	30	1.6	5.3	88.9
Total	12.8	1.5	11.7	92.8	22.2	1.4	6.2	92.8

GLM Analysis. GLM analysis revealed that the type of nest, type of plant, height from ground level, and distance to nearest house were all significantly associated with the quantity of triatomines found in the sampled microhabitats (Table 3; $P < 0.001$). Squirrel nests, located in plants other than palms, above 5 m and close to the domicile, presented highest infestation with triatomines. The removal of the factor of distance to the nearest house had the highest change in the AIC value, being the parameter responsible for most of the observed variation.

Fig. 4 presents the relationship between the abundance of triatomines in the two study sites within ranges of 100-m distances and the distance to the nearest house. The Lorentzian cumulative function ($F = 81.52$, $R^2 = 0.958$) predicted an 82% decrease in the total number of triatomines at a 300-m distance from the nearest house. From 300 to 600 m from the house, the abundance of triatomine has a continuous decrease, and from 600 to 1,000 m from the nearest house there is a marked decrease in triatomine abundance, having few or almost no triatomines.

Density Interpolation. As expected, the interpolation maps of El Bejuco and Maconta Abajo communities predicted a high triatomine density in areas where the insects were found aggregated and places nearby (Fig. 5), mostly located close to the domiciles. A lower density of triatomines was predicted in other sylvatic areas of the quadrat.

Discussion

Sampling Design. The limited information available on the ecology of sylvatic Triatominae is partly because of the difficulty in sampling these insects (Noireau et al. 2005). Despite some positive results obtained by other investigations in the use of live-baited traps for triatomine collection (Noireau et al. 2002, Abad-Franch et al. 2005), especially when placed in palm trees, our results showed a low efficacy of these traps in the sampled microhabitats. This low efficacy in our study could be because of the random selection of points and the unbiased selection of microhabitats within each point. In addition, triatomine are known for their relatively low mobility especially in well-fed populations (Schofield 1994, Patterson 1979). Therefore, although labor intensive, manual searches appeared to be the most productive method for triatomine collection in Ecuadorian Coastal habitats.

The randomized methodology designed in our study lessens sampling bias, as several microhabitats are sampled and the time and sampling effort can be standardized. In addition, it imposes sampling standardization allowing comparison of results from different sites, which is an important caveat of directed manual search methods that are often highly dependent on searcher's experience. Another important benefit of this design is obtaining unbiased population and nutritional status data as was observed in the sampled microhabitats (data not shown). This random sampling method was implemented as a pilot study to determine the distribution of sylvatic triatomines. Because of the small spatial scope used in this research, there is a need of further studies that include more comprehensive surveys that validate the patterns found and the use of more efficient sampling methods.

Vectors of Chagas Disease in Ecuador. Although *T. dimidiata* is considered to be the main Chagas Disease vector species in the Coastal region of Ecuador (Abad-

Table 2. Infestation and no. of triatomines collected in the different types of nests and plants checked in the two communities sampled in Manabí province

Microhabitat	No. microhabitats checked	No. positive microhabitats*	% positivity	No. triatomines collected	Mean bug density per positive nest or plant (SD)
Nests					
Bird nest	77	6	7.8	131	21.8 (6.6)
Mouse nest	145	33	22.8	28	0.8 (3.5)
Squirrel nest	30	17	56.7	185	10.9 (12.1)
Total	252	56	22.2	344	6.1 (8)
Plants					
Palm	49	30	61.2	143	4.8 (4.7)
Other plants	325	47	14.5	202	4.3 (10.1)
Total	374	77	20.6	345**	4.5 (8)

* Microhabitats positive for triatomine presence.
** Additional triatomine collected by a live-baited trap in a *P. aequatorialis* palm.

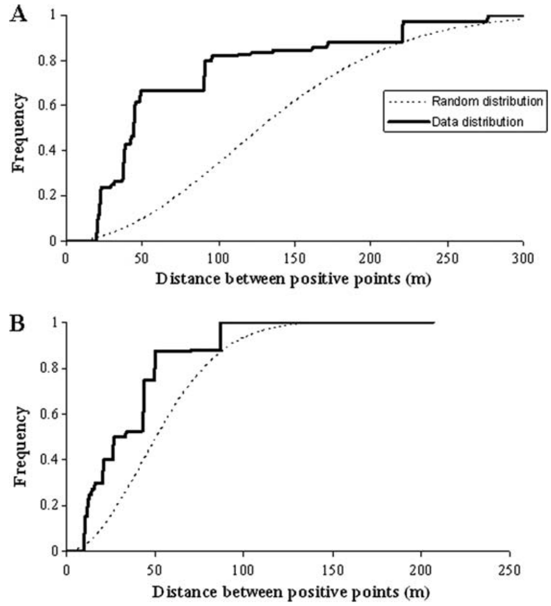

Fig. 3. Values of the nearest neighbor distance function $G(r)$ related to the distance between positive triatomine sampling points in the sylvatic quadrat in (A) El Bejuco and (B) Maconta Abajo. Dashed line represents the null model of spatial randomness.

Franch et al. 2001), extensive surveys conducted in this area from 2004 to 2007 did not find any *T. dimidiata* specimens, perhaps as a result of the insecticide-based interventions applied in this region for the control of mosquitoes and triatomines. In contrast, since 2004, large peridomiciliary colonies and occasional intradomiciliary invasion by *P. howardi* have been found in the Portoviejo River Valley in Manabí province (M. J. Grijalva, unpublished data). Despite the emerging importance of this species, little is known about its ecology and life cycle.

An important finding of this study is the occurrence of sylvatic specimens of *P. howardi* associated with mouse nests in the palm *A. eggersii* and the terrestrial bromeliad *B. pinguin.* To our knowledge, this is the first published report of *P. howardi* in a sylvatic habitat. *P. howardi* specimens have been found in all stages of

Table 3. Results of the generalized linear model's analysis on triatomine sampling

Effect	Terms included in the model	AIC	ΔAIC	*P* value
Nest	All	1006.4	138.8	<0.001
Plant	All	1016.1	148.5	<0.001
Height	All	1039.1	171.53	<0.001
Distance	All	1062.5	194.93	<0.001

AIC is given to test for the significance of selected ecological factors on triatomine infestation. Likelihood ratio test was used to find the difference between the initial model and the reduced model, dropping an "effect" term. Likelihood ratio test and corresponding *P* value test the hypothesis that the suppression of the effect term provides no significant better fit than the initial model.

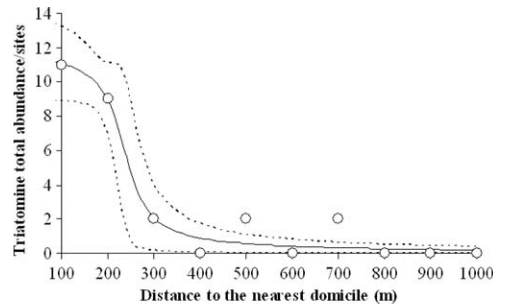

Fig. 4. Abundance of triatomine in the sampled sites related to ranges of distance to the nearest house of 100 m.

growth, near nests of rats, mice and bullfrogs (*Rana catesbeiana*) in peridomiciliary habitats, and have been found infected with *T. cruzi* (M. J. Grijalva, unpublished data). Although this species has previously been reported in this region (Abad-Franch et al. 2001), its dorsal coloration resembles that of *T. dimidiata* and close examination of the head is required for accurate identification. Possibly its emerging importance in the region has been underreported by local surveillance efforts. The occurrence of *P. howardi* in the peridomicile area highlights the need of further ecological and epidemiological studies of this species. In addition, in palm searches conducted outside of the quadrats in Maconta Abajo one *P. rufotuberculatus* third stage nymph was found in a mouse nest located in a *P. aequatorialis* palm. The finding of *P. howardi* and *P. rufotuberculatus* specimens associated with palms is very important to identify these species' sylvatic habitats.

Our data suggest that sylvatic populations of *R. ecuadoriensis* found in the sampled quadrats are well established and reproducing within themselves as a considerable number of nymphal stages was found. The quantity of specimens found emphasizes its importance as *T. cruzi* vector in coastal areas of the country.

T. cruzi infection was found both in *R. ecuadoriensis* and *P. howardi* collected in peridomestic habitats and in *R. ecuadoriensis* collected in sylvatic habitats, highlighting the importance of these two species as Chagas disease vectors. In addition, previous studies found a 5.7% Chagas disease seroprevalence in nearby communities located in Manabí province (Black et al. 2007). Moreover, recent serological surveys conducted in collaboration with the National Chagas Control Program have found pediatric cases in the Portoviejo River Valley (M. J. Grijalva, unpublished data), which highlights the need for studies that provide more information regarding the ecology of the local *T. cruzi* vectors.

Spatial Distribution of Triatomines. Spatial distribution data are important for informed decision-based vector control interventions (Bustamante et al. 2007). Besides the high-peridomiciliary infestation, our results showed a significant triatomine aggregation (<100 m) in both peridomiciliary and sylvatic areas. These results coincide with studies of the distribution

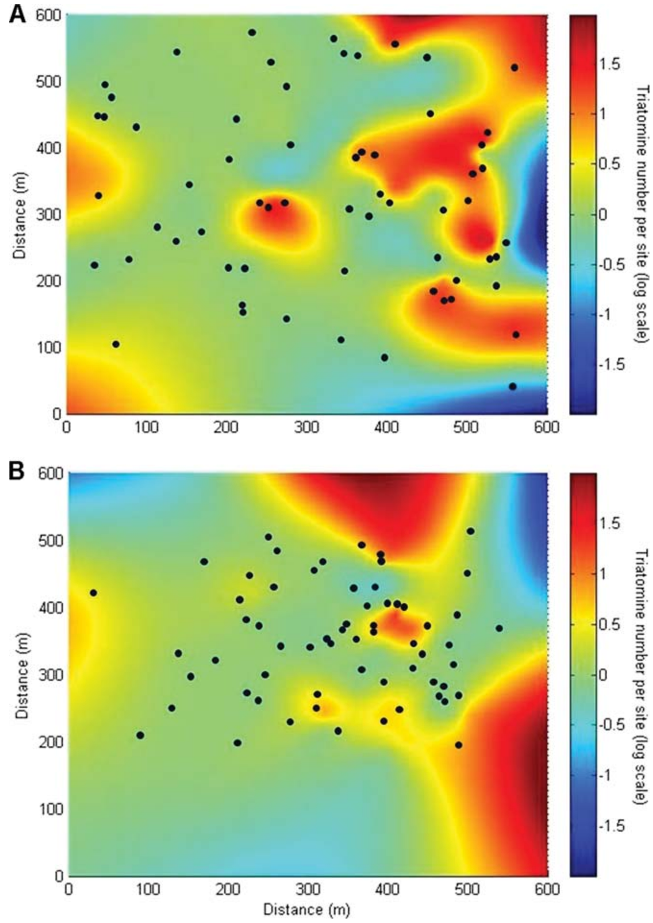

Fig. 5. Triatomine density interpolations in sylvatic areas near the communities studied in the province of Manabí. Areas with more triatomines are presented with reddish colors, while areas with few or no triatomines are in blue (see the color log-scale of densities). Spots represent the randomly generated points sampled in the quadrat. Nearest houses were located 100–200 m from the right side of both maps. Predictive map obtained for the community of (A) El Bejuco and (B) Maconta Abajo. (Online figure in color.)

of domestic triatomine populations showing aggregation in domicile and peridomicile areas (Vazquez-Prokopec et al. 2005). The low mobility and active dispersal of triatomines (Schofield 1994) could explain the aggregation found, as triatomines rest near their feeding source and maintain low activity during the day (Lorenzo Figueiras et al. 1994). Some studies also suggest that there are chemical substances in feces that facilitate population aggregations, because these substances are associated with safe resting places and nearby feeding sources (Lorenzo and Lazzari 1996). Because our results show that local triatomine distribution is significantly aggregated and not random, entomological surveillance should be extended to areas within a 300-m radius around a given positive location to monitor sylvatic triatomine populations that could serve as sources of reinfestation.

Ecological Determinants of Triatomine Distribution. This investigation allowed the identification of some risk factors associated with high triatomine infestation in sylvatic areas of Manabí province. Environmental variables as temperature, relative humidity, and altitude are known to influence triatomine distribution at a regional level (Bustamante et al. 2007), but they are not the main determinants for triatomine microhabitat distribution. We found a significant correlation between triatomine density and the following factors: squirrel nests, at a height of more than 5 m, and close to human dwellings.

Besides the known association of *R. ecuadoriensis* with nests in palms (Noireau et al. 2002, Abad-Franch et al. 2005), it is also found in nests located in a variety of plant species. The plant type may affect triatomine microdistribution inherently by providing harborage for warm blood animals that serve as triatomine hosts (Gorla 2002). The relatively large body size of squirrels and the characteristics of their nests (large, ≈30 cm diameter, loose construction, and usually located at >5 m from the ground) could improve food source availability and protection for triatomines. The collection of triatomines in nests located at 5 m or more can be explained by the dispersal flight by host-seeking adult triatomines (Vazquez-Prokopec et al. 2004).

Finally, the distance to the domicile was most strongly correlated with triatomine densities, even though the presence of host nests was evenly distributed in the sampled areas. This may be explained by the presence of opportunistic feral mammals that are attracted to crops and food sources that result from anthropogenic activities. These animals could serve as food meal for triatomines and attract them near the houses. Although no intradomiciliary infestation was observed, perhaps because of previous spraying, as the residual effect of deltamethrin lasts at least 3 mo (Rojas de Arias et al. 2004), the level of peridomiciliary infestation indicates a strong synanthropic tendency in both species. Further, triatomine density maps predicted patches of high triatomine abundances near the domicile, which could favor the introduction of sylvatic *T. cruzi* into human hosts.

Our results regarding the microdistribution of sylvatic triatomines indicate that although the entomological surveillance of Chagas disease control programs should focus in domestic and peridomestic habitats with high triatomine infestation, surveillance efforts should include nearby sylvatic habitats. The triatomine microdistribution information we obtained highlights the challenges faced by Chagas control programs in the region and strongly supports the need for continuous active and passive entomological surveillance, also suggesting that insecticide-based control by itself may not suffice for the long-term prevention of triatomine-human contact.

Further studies are being conducted using molecular and morphometric techniques to determine the relationship and gene flow direction between synanthropic and sylvatic populations of *R. ecuadoriensis.* However, given the history of vector control in the area and the results of our spatial analyses, together with the high number of adult specimens found in the peridomicile, it seems likely that the sylvatic populations contribute significantly to peridomicile infestation.

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