Journal of Medical Entomology

Seasonality and temperature-dependent flight dispersal of Triatoma infestans and other vectors of Chagas disease in western Argentina

Journal:	*Journal of Medical Entomology*
Manuscript ID	JME-2017-0026.R2
Manuscript Type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Di Iorio, Osvaldo; Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Biodiversidad y Biología Experimental Gürtler, Ricardo; University of Buenos Aires, Ecología, Genética y Evolución; Consejo Nacional de Investigaciones Científicas y Técnicas. , Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBA),
Please choose a section from the list:	Sampling, Distribution, Dispersal
Field Keywords:	Medical Entomology, Trypanosomiasis, Chagas Disease, Vector Ecology, Surveillance
Organism Keywords:	Triatominae

Di Iorio and Gürtler.

Seasonal flight dispersal of Chagas disease vectors.

Sampling, Distribution, Dispersal

R. E. Gürtler
Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBA), Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina.
Tel/fax: (5411) 4576-3318
E-mail: gurtler@ege.fcen.uba.ar

Seasonality and temperature-dependent flight dispersal of *Triatoma infestans* and other vectors of Chagas disease in western Argentina

Osvaldo Di Iorio,[1,†] and Ricardo E. Gürtler[2,3]

[1] Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales, Departamento de Biodiversidad y Biología Experimental, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina, and † deceased.

[2] Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales, Departamento de Ecología, Genética y Evolución, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina.

[3] Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad de Buenos Aires. Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBA), Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina.

Abstract

Flight dispersal of Triatominae is affected by climatic conditions and determines the spatiotemporal patterns of house invasion and transmission of *Trypanosoma cruzi* Chagas (Kinetoplastida: Trypanosomatidae). We investigated the detailed time structure and temperature dependencies of flight occurrence of *Triatoma infestans* Klug (Hemiptera: Reduviidae) and other triatomine species in a rural village of western Argentina by taking advantage of the attraction of adult triatomines to artificial light sources. Most of the village's streetlight posts were systematically inspected for triatomines twice between sunset and midnight over 425 nights in the spring-summer seasons of 1999-2002, an unprecedented light-trap sampling effort for any triatomine species. A total of 288 adults were captured, including 122 *Triatoma guasayana* Wygodzinsky and Abalos, 89 *T. infestans*, 72 *Triatoma eratyrusiformis* Del Ponte and 5 *Triatoma garciabesi* Carcavallo et al. Adult sex ratios were balanced in *T. infestans* and strongly male-biased in other species. Nearly all flight-dispersing triatomines were caught when temperatures at sunset were above 20°C (range, 16.6-31.7°C), suggesting a putative threshold around 17-18°C. Triatomine catches were rare on rainy days. Logistic regression analysis revealed that the proportion of nights in which at least an adult *T. infestans* was caught increased highly significantly with increasing temperature at sunset and was modified by collection month, with greater catches in early spring and no sex differential. This study confirms that spring represents a previously overlooked, important dispersal period of *T. infestans*, and shows large variations among and within Triatominae in their temporal patterns of flight occurrence, abundance and sex ratio.

Key words *Triatoma guasayana*, *Triatoma eratyrusiformis*, *Trypanosoma cruzi*, vector control, temperature, weather

Active dispersal of Triatominae (Hemiptera: Reduviidae) determines the spatial patterns of house invasion and vector infection with *Trypanosoma cruzi* Chagas (Kinetoplastida: Trypanosomatidae), the etiologic agent of Chagas disease (Vazquez-Prokopec et al. 2006, Levy et al. 2008, Barbu et al. 2011). These patterns, derived from environmental conditions combined with nutritional and reproductive status, modify the flight activity of triatomines (Sjögren and Ryckman 1966, Ekkens 1981, Lehane et al. 1992, Schofield et al. 1992, Gurevitz et al. 2006). For example, higher triatomine densities per host would impair the insects' nutritional status (measured by body weight-to-length ratios, W:L), increase the likelihood of flight initiation, and contribute to the regulation of local population density (Lehane et al. 1992, Schofield et al. 1992). Artificial light sources usually attract adult triatomines of many species and favor house invasion (Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2006, Minoli and Lazzari 2006, Carbajal de la Fuente et al. 2007, Castro et al. 2010, Abrahan et al. 2011, Pacheco-Tucuch et al. 2012, Jácome-Pinilla et al. 2015).

Seasonal variations in flight dispersal are fairly common in Triatominae, with flights occurring within the first 2-3 h after sunset in most of the species investigated in the field (Sjögren and Ryckman 1966, Schofield et al. 1992, Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2006). For example, the flight activity of *Triatoma protracta* Uhler and *Triatoma rubida* Neiva peaked in summer in the Arizona desert (Sjögren and Ryckman 1966, Ekkens 1981), whereas flights of *Triatoma dimidiata* Latreille and other species peaked in late spring (dry season) in the forests of Costa Rica (Zeledón et al. 2001). Catches of nine species of Triatominae in a light trap located at canopy level peaked in October-December in the Amazon rainforest (Castro et al. 2010). *Triatoma guasayana* Wygodzinsky and Abalos, a sylvatic or peridomestic species in the dry Chaco ecoregion extending over sections of Argentina, Bolivia and Paraguay, apparently had a distinct flight dispersal window in spring (Gürtler et al. 1999, Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2008, Abrahan et al. 2011, Ceballos et al. 2011), but light-trap efforts at other times are rather sparse.

The flight initiation probabilities of *Triatoma infestans* Klug, the vector responsible for the majority of human infections with *T. cruzi*, significantly decreased with increasing W:L ratios and increased with ambient temperature (Lehane et al. 1992, Schofield et al. 1992, Gurevitz et al. 2006). Light-trap surveys and cage experiments under natural climatic conditions showed that *T. infestans* initiated flight between 22 and 23°C (Vazquez-Prokopec et al. 2006, Gurevitz et al. 2006), whereas in a laboratory setting flight initiation was rare at 18°C and then increased steeply between 20 and 30°C

(Lehane et al. 1992). Whether there is a threshold temperature at which triatomines initiate flight and the functional relationship between flight activity and ambient temperature merit further investigation.

The flight dispersal rate of *T. infestans* was predicted and observed to peak in late summer in the dry Argentine Chaco, when adult triatomines also peaked in abundance and reached minimal W:L ratios (Ceballos et al. 2005, Vazquez-Prokopec et al. 2006). However, a detailed analysis of the spatio-temporal patterns of site infestation in this rural area over 10 years (zu Dohna et al. 2009) and a habitat-specific flight index supported that early spring may represent an important dispersal period of *T. infestans* which previous limited light-trap efforts had not detected (Gürtler et al. 2014). Thus, the precise time window at which flight dispersal occurs and potential differences between sexes (Ekkens 1981) remain unknown. This knowledge may be used for modeling house invasion and colonization (e.g. Crawford and Kribs-Zaleta 2013) and has implications for the optimal timing of vector control actions.

Asystematic survey of most of the public streetlights of a small rural village in western Argentina was conducted between sunset and midnight over three consecutive spring-summer seasons (Di Iorio 2014). This unprecedented light-trap sampling effort for triatomines yielded a sizable number of specimens of *T. infestans, T. guasayana, Triatoma eratyrusiformis* Del Ponte and *Triatoma garciabesi* Carcavallo et al. We used this information to investigate whether spring and summer may represent important dispersal periods of *T. infestans* and *T. guasayana*, respectively, and the relationship between flight dispersal, temperature at sunset and triatomine collection month.

Materials and methods

Study area. Field work was conducted at Los Molinos (28° 43' 60'' S, 66° 55' 60'' W), a small rural village with approximately 600 people located at 1,254 m above sea level in an arid zone of La Rioja province (Departamento Castro Barros), western Argentina. The village was situated on low terrain, which determined the virtual absence of strong winds. Most of Los Molinos village comprised abandoned orchards with artificial irrigation and houses with well-plastered brick walls and tin, cement or tiled roofs. Householders usually raised chickens, goats, dogs and pigs. Estates were separated by old fences made with piled rocks. High-pressure mercury-vapor lamps (250 watts, which radiate predominantly in the ultraviolet and visible regions of the optical range) were used for street lighting. Mercury vapor lamps are energetically more efficient than incandescent and most fluorescent lights, with luminous efficacies of 35 to 65 lumens/watt (i.e., a high-intensity, clear white light output). Local residents reported that Chagas vector control programs had not sprayed their houses with insecticide between 1991 and 2002. The local climate was markedly seasonal, with dry (fall-winter) and wet (spring-summer) periods.

Mean monthly temperatures at sunset increased from 19.9°C in October to 23.1-23.6°C between December and February, with substantial variations within the same month over the study period (Di Iorio 2014). Monthly temperatures during the second summer (December 2000-February 2001) were on average 2-3°C higher than in the previous or subsequent years. Monthly minima at sunset ranged from 11.0 to 13.3°C between October and December, and increased to 16.7-17.3°C between January and March throughout the study period. The percentage of days with rainfall was much less frequent in October (mean, 7.5%; range, 0.0-12.9%) or November (10.8%; 9.7-12.9%) than in December (25.8%; 12.9-41.9%), January (36.6%; 32.3-41.8%) and February (25.8%; 19.4-32.3%). The second spring-summer period (2000-2001) was unusually wet relative to the previous and subsequent years, with much greater herbaceous coverage and insect abundance in several areas.

Study design. A longitudinal study was conducted during three consecutive spring-summer seasons (October 1999-January 2000, October 2000-February 2001, and October 2001-March 2002) using a systematic survey design (Di Iorio 2014). Every survey night all 29 mercury-vapor lamps were systematically and sequentially visited twice between sunset and midnight over a total of 425 nights. Each sampling round was organized into five pathways. Because some sections of the village had no alternative access, 14 light posts were inspected twice in each round: one on the way to the end of the street and another on the way back. The ground, vegetation, light poles and other suitable places located within and adjacent to the light cone below each lamp were visually inspected for insects within a 5-15 min sampling period by the senior author. All insects of interest were collected manually, put in killing jars, and later transferred to storage boxes. All collected specimens were subsequently re-examined for an independent corroboration and identified to species following Wygodzinsky and Abalos (1950) and Lent and Wygodzinsky (1979) and nomenclature proposed by Galvão et al. (2003) and Sainz et al. (2004).

Temperatures were recorded exactly at sunset with a digital thermo-hygrometer, except in October 1999 and February 2000, and with a red-colored alcohol thermometer in November and December 1999 for a total of 425 days. These data were compared with those collected by a weather station at La Rioja airport at 500 m of altitude, approximately 200 km from Los Molinos village (Servicio Meteorológico Nacional, unpublished data). Both data sets showed an average difference of 6.8°C at sunset (N = 392 observations) and therefore the airport weather station data were excluded from further consideration.

Data analysis. The database included 425 daily observations on the species-specific occurrence and number of triatomines caught at the streetlights, of which 394 also had records of temperatures at sunset and rainy weather. Maximum-likelihood logistic regression was used to assess the effects of temperature at sunset (a continuous variable) and triatomine collection month (a categorical variable) on the catch of at least one *T. infestans* on a particular night (binary response variable). Triatomine collection month had five levels representing January, February, November and October relative to December, taken as the reference level. This analysis excluded March (when no triatomine was collected); pooled data over year of collection to avoid small-sample bias, and included 289 observations with complete data on the selected predictors. We verified whether there was any non-linear effect of temperatures at sunset by adding a squared term, which was non-significant for the three species, and run separate regressions for males and females to assess whether model coefficients were significantly different. The goodness of fit of the model was assessed by the Hosmer-Lemeshow test and the area under the Receiver Operating Characteristic (ROC) curve by using 10 quantiles. All analyses were run on Stata 14.2 (StataCorp 2017) using commands *logistic*, *estat gof* and *lroc*. The same analysis was conducted for *T. guasayana* and *T. eratyrusiformis*; catches of *T. garciabesi* were sparse and, therefore, were excluded from detailed analysis. The co-occurrence of male and female adults of a triatomine species on a particular night was tested with a two-sided exact McNemar's test.

Results

A total of 288 specimens of four species of Triatominae were captured at the streetlight posts between 1999 and 2002 (Table 1). Adult triatomines were spotted resting on the substrate or walking within the light cone when they were captured. *Triatoma guasayana* was the most abundant species, representing 42.4% of the total catch, followed by *T. infestans* (30.9%) and *T. eratyrusiformis* (25.0%). Only 5 (1.7%) specimens of *T. garciabesi* were caught. This ranking also held for the average catch per trap-night and the frequency of sample nights in which each species was collected: *T. guasayana* was caught in 75 (17.6%) of 425 sample nights, closely followed by *T. infestans* (14.1%), *T. eratyrusiformis* (12.0%), and *T. garciabesi* (1.2%). Overall adult sex ratios were fairly balanced in *T. infestans* (47.2% of females) and highly skewed toward males in the other species. All specimens were unfed when they were captured as confirmed by visual inspection.

Most of the catches of *T. infestans* comprised only one insect (on 44 occasions); 2, 3, 4 and ≥5 insects were collected during the same night on 9, 3, 2 and 2 occasions, respectively. The frequency distributions of 1, 2, 3, 4 and 5 adult triatomines collected were 50, 15, 4, 1 and 5 for *T. guasayana*, and 37, 9, 3, 2 and 0 for *T. eratyrusiformis*, respectively. Male *T. guasayana* and *T. eratyrusiformis* were caught significantly more often than females of the corresponding species during the same night, but this relationship did not hold for *T. infestans* (Table 2).

Most adults of *T. infestans* were collected over the second spring-summer season (53 of 89, 60%), with peak catches at October and February (Fig. 1A). No catches occurred in October and few in November-December of the first spring-summer season, whereas nearly all catches occurred between October and December of the third season. The overall adult sex ratio displayed substantial variations among months (Fig. 1A). Pooling monthly data over sample years, the female-to-male ratio (female:male) favored males in October (7:15), females in November (9:6) and December (12:6), neither sex in January (9:10), and favored males again in February (5:10).

Nearly all catches of flight-dispersing triatomines (282 of 288, 97.9%) occurred when temperatures at sunset were above 20°C (range, 16.6-31.7°C) (Fig. 2A-C). Only in one (3%) of the 92 rainy days recorded throughout the study was an adult triatomine caught at the streetlights. For *T. infestans*, the minimum temperature at sunset at which an adult triatomine was captured was 16.6°C (in October) and the maximum was at 31.7°C (in February) (Fig. 2A). The logit of the proportion of sample nights in which at least an adult *T. infestans* was caught on a non-rainy day (y) increased significantly with temperature at sunset (x_1) and was modified by collection month of insect (x_{2-5}) ($y = 0.4312$ (SE, 0.0685) $x_1 - 0.2617$ (SE, 0.5319) $x_2 - 0.5706$ (SE, 0.4977) $x_3 + 0.2515$ (SE, 0.4947) $x_4 + 1.4126$ (SE, 0.5593) $x_5 - 11.8796$ (SE, 1.7756); $\chi^2 = 63.06$, df = 5, $P < 0.0001$, pseudo $R^2 = 0.216$), where x_{2-5} represent January, February, November and October, respectively, taking December as the reference level. The temperature-adjusted relative odds of a catch was fourfold greater in October than in December (OR = 4.11, 95% confidence interval, 1.37-12.29). The model displayed a close fit to the data (Hosmer-Lemeshow $\chi^2 =$ 5.66, 8 df, $P = 0.685$), with total observations for each quantile ranging from 26 to 33. The area under the ROC curve was 0.815. Model coefficients were not significantly modified by sex of insect. Figure 3 shows the observed and predicted percentage of nights with a catch (both sexes combined) as a function of temperatures at sunset between 15 and 31°C; only two data points at both extremes deviated significantly from predictions.

For *T. guasayana*, nearly 50% of all specimens were caught during the second season (Fig. 1B).

The flight period spanned from October to December, with very few specimens collected from January to February and peak catches in October. The overall sex ratio was consistently male-biased. The minimum and maximum temperatures at which specimens were captured were 19.4 and 30.7°C, respectively (Fig. 2B). The logistic regression model displayed a close fit to the observed proportion of nights in which at least a *T. guasayana* was caught (Hosmer-Lemeshow $\chi^2 = 4.91$, 8 df, $P = 0.767$), and the area under the ROC curve was 0.817 ($y = 0.3661$ (SE, 0.0605) $x_1 - 2.7058$ (SE, 0.8237) $x_2 - 1.3746$ (SE, 0.5198) $x_3 +$ 0.9211 (SE, 0.4362) $x_4 + 1.3365$ (SE, 0.5107) $x_5 - 9.8437$ (SE, 1.5477); $\chi^2 = 70.67$, df = 5, $P < 0.0001$, pseudo $R^2 = 0.224$).

For *T. eratyrusiformis*, the flight period also extended from October to December, with peak catches in November-December and very few specimens collected over January and February (Fig. 1C). The overall sex ratio was male-biased throughout the observation period. The minimum and maximum temperatures at which specimens were captured were 19.4 and 29.8°C, respectively (Fig. 2C). As with the other species, the logistic model displayed a close fit to the data (Hosmer-Lemeshow $\chi^2 = 10.46$, 8 df, $P =$ 0.234), and the area under the ROC curve was 0.844 ($y = 0.3415$ (SE, 0.0665) $x_1 - 1.9035$ (SE, 0.7123) $x_2 - 3.2070$ (SE, 1.0640) $x_3 + 0.8151$ (SE, 0.4513) $x_4 - 0.2610$ (SE, 0.6110) $x_5 - 9.5031$ (SE, 1.7074); $\chi^2 = 60.49$, df = 5, $P < 0.0001$, pseudo $R^2 = 0.242$).

Discussion

The most important results of our study are related to the seasonal timing of flight dispersal in *T. infestans* and other triatomines, and the close relationship between flight occurrence toward streetlights and temperatures at sunset. Our study documents a large number of dispersing adults of *T. infestans,* which were mainly collected during spring of the second year (the warmest over the 3-yr period), with greater catches in October. This pattern is consistent with the outcomes of a detailed time-series analysis of site occupancy of *T. infestans* over 10 years (zu Dohna et al. 2009), and habitat-specific flight indices based on blood-feeding patterns and other population attributes in the dry Chaco mid-spring (Gürtler et al. 2014). These patterns were not supported by other light-trap surveys with sparse sampling during spring (Vazquez-Prokopec et al. 2006, Abrahan et al. 2011). Taken together, the evidence strongly refutes that flight dispersal of *T. infestans* is rare in spring. We note, however, that weather conditions (including temperatures at sunset) were different in Los Molinos, situated in arid western Argentina at 1,200 m of altitude, than in the dry Chaco region of Santiago del Estero at less than 200 m of elevation. Insect flights at dusk declined steeply during March at the higher altitude of Los Molinos (Di Iorio, unpublished data), unlike in the dry Chaco studies. The spring flight dispersal window is likely to be caused by a chain of events associated with rising spring temperatures and increasing day length after the cold winter months in which triatomines remained metabolically inactive, followed by peak adult emergence (Gorla and Schofield 1980) and the 3- or 4-wk period needed for flight muscle development (Gurevitz et al. 2006). Spring flights are finally made evident under suitable weather conditions.

The occurrence of an apparent spring flight window in *T. infestans* does not alter the fact that flight dispersal is more likely to be recorded during the warmer summer months than on the cooler spring months. This statement is contingent upon the weight-to-length (W:L) distribution of triatomine populations, which itself suffers large seasonal and habitat variations (Ceballos et al. 2005). The large inter-annual variability of weather conditions in the Gran Chaco is similar to the large weather variations recorded in Los Molinos over a limited 3-yr period.

Nearly all specimens of the four triatomine species flew at temperatures at sunset above 20°C, suggesting a putative threshold for flight dispersal around 17-18°C. This estimate is consistent with experimental evidence showing that the spontaneous locomotory activity of male *T. infestans* would be strongly reduced below 20°C (Lazzari 1992), whereas flight initiation steeply increased from 0% at 15°C, 6.2% at 18°C to 35.2% at 22°C (after pooling data for both sexes and repeat experiments at given temperatures in Table 1 of Lehane et al. 1992). Such thresholds may be related to the activity of the two isoforms of the enzyme glycerol-3-phosphate dehydrogenase, which is crucially linked to flight metabolism in triatomines (Stroppa et al. 2013).

The relative odds of flight occurrence of *T. infestans* toward the streetlights increased highly significantly with increasing temperature at sunset in both sexes. Similarly, flight initiation probabilities increased sigmoidally with increasing temperature at sunset in caged experimental huts under natural climatic conditions, although females displayed consistently higher flight probabilities than males after adjusting for W:L effects (Gurevitz et al. 2006). Both metrics are intimately linked by the same temperature-dependent, binomial process: flight initiation probabilities most likely generated the proportions of nights in which at least one *T. infestans* flew toward the streetlights. In the experimental huts, the asymptote recorded over 25-30°C was in part determined by the large fraction of adult triatomines lacking flight muscles (Gurevitz et al. 2007) whereas in the current study there was no asymptote. Here the proportion of females among flight-dispersing adults of *T. infestans* (47.2%) was substantially larger than in light-trap surveys conducted in Santiago del Estero (33.3% of 21 adults) (Vazquez-Prokopec et al. 2006) and in La Rioja (37.5% of 8 adults) (Abrahan et al. 2011). For comparison, the mean percentage of adult females over domestic and peridomestic populations of *T. infestans* averaged 41.7% and varied substantially among ecotopes in the mid-spring of Santiago del Estero (Gürtler et al. 2014).

Our study documents different patterns of flight occurrence, abundance and adult sex ratios among the four species of Triatominae investigated, with large variations among and within years for any one species running in parallel to wide variations in weather conditions. The four triatomine species were most frequently collected at the streetlights during the second (2000-2001) summer period, which was substantially warmer and wetter than the previous or subsequent summers, and only nine (3.1%) of all triatomines collected were caught on a rainy day. In addition, males of *T. guasayana* and *T. eratyrusiformis* occurred significantly more frequently than females of each species, rejecting the hypothesis of independence between sexes. These male-biased patterns may perhaps be explained by pheromone-mediated searches for mates, with an increasing frequency of male fliers in response to female pheromones (Zacharias et al. 2010), and he lower probabilities of flight dispersal of gravid females.

Triatoma guasayana was the species most frequently collected at the streetlights, in agreement with previous light-trap and manual-search surveys in the dry Argentine, Bolivian and Paraguayan Chaco regions (Noireau and Dujardin 2001, Vezzani et al. 2001, Yeo et al. 2005, Vazquez-Prokopec et al. 2008, Ceballos et al. 2011). *Triatoma guasayana* usually occurs in sylvatic conditions under the loose bark of trees or in rocks, fallen trees, dry cacti, bromeliads and logs, and also colonizes goat or sheep corrals, piled materials, and orchard fences (Canale et al. 2000, Vazquez-Prokopec et al. 2008, Di Iorio and Turienzo 2011). In contrast to female-biased or balanced adult sex ratios (Noireau and Dujardin 2001, Ceballos et al. 2011, Abrahan et al. 2011), the current data show a strong predominance of *T. guasayana* males over seasons and years.

Triatoma eratyrusiformis, a large species found in arid western Argentina, was less abundant and appeared slightly later than *T. guasayana* during spring. The former species is sometimes collected in domestic or peridomestic premises (Lent and Wygodzinsky 1979, Di Iorio and Turienzo 2011), and unlike *T. guasayana* and *T. garciabesi*, *T. eratyrusiformis* is frequently infected with *T. cruzi* through its association with peridomestic rodents (Cecere et al. 2016).

Triatoma garciabesi was rarely collected at the streetlight posts. This species is frequently found in chicken coops, bird nests and in the rugged bark of *Prosopis* trees where chickens roosted (Canale et al. 2000, Turienzo and Di Iorio, 2014, Rodríguez-Planes et al. 2016). Although *T. garciabesi* outnumbered *T. guasayana* in the peridomestic habitats of Santiago del Estero over time (Canale et al. 2000, Rodríguez-Planes et al. 2016), the former was rarely collected using light traps or by householders as part of routine vector surveillance; none of them colonized human sleeping quarters (Gürtler et al. 1999). Therefore, the marginal light-trap catches of *T. garciabesi* in the study village and elsewhere in La Rioja (Abrahan et al. 2011) may not necessarily imply low abundance in the surrounding habitats. The rare appearance of *T. garciabesi* in light-trap catches and in householders' triatomine collections is in stark contrast with the frequent flight behavior of *Triatoma sordida* Stål (Schofield et al. 1991) despite the former being a cryptic species within the *sordida* complex (Gurgel-Gonçalvez et al. 2011).

The interpretation of our results is limited by lack of additional information. Data on wind speed may be important for more accurate predictions of flight occurrence because winds greater than 5-6 km/h apparently discourage the initiation of flight in *T. infestans* under natural conditions (Vazquez-Prokopec et al. 2006). In Los Molinos, however, its low-land location reduced the frequency of strong winds. Adult triatomines most likely flew toward the high-intensity white lights (Minoli and Lazzari 2006), as expected from their unfed status, but some may have walked from nearby sources. The village did not undergo any major disturbance and most likely remained infested at rather similar levels throughout the study period, but local triatomine abundance and the nutritional status of the putative source populations were not investigated. Given the lack of government-sponsored house spraying with insecticide during the previous decade, a reasonable assumption is that *T. infestans* population density fluctuated seasonally around average values. The rest of the triatomine species collected in this study have extensive sylvatic and peridomestic foci and, therefore, were less likely to be affected than *T. infestans*. The current study covered a wide range of climatic conditions over three consecutive spring-summer seasons, but sample periods within any one year did not completely overlap and end-of-summer data (March) were sparse. The extent of the sampling effort did not allow more detailed observations at the level of individual streetlights; local effects were apparent, with few light posts yielding most catches. The systematic search of streetlights provided ample coverage across a well-defined area over several spring-summer seasons, which constitutes an unprecedented light-trap sampling effort of triatomines.

Our current results have implications for improved vector and disease control and modeling of house invasion via flight dispersal: i) adult triatomines of various species attracted to public streetlights are more likely to invade the neighboring houses, leading to spatial clustering of infestation in the vicinities of artificial light sources, as reported for *T. dimidiata* in Yucatan (Pacheco-Tucuch et al. 2012) and *Rhodnius prolixus* Stål in Colombia (Jácome-Pinilla et al. 2015). Here we show the same pattern is applicable to *T. infestans* in a small rural town. Because adult stages typically display the largest prevalence rates of *T. cruzi* infection, such house invasions may ensue human infection risks; ii) analysis of spatiotemporal infestation patterns suggested that *T. infestans* may disperse further than 1.5 km in open fields (zu Dohna et al. 2009), whereas *T. dimidiata* and *R. prolixus* would be attracted by streetlights up to 100 m and by house lights at least for 110 m, respectively (Barbu et al. 2010, Jácome-Pinilla et al. 2015). Therefore, the exact sources of the dispersing triatomines may be quite distant to the target streetlights depending on terrain features and vegetation cover; iii) The predicted increase in minimum temperatures fueled by climate change is expected to widen the time window for triatomine flight dispersal, and most likely increase the rates of house invasion; iv) the relationship between temperatures at sunset and flight occurrence combined with local weather information may be used to predict the frequency of house invasion with *T. infestans* at different locations. The time window for flight dispersal was most likely restricted to the October-March period through most of the dry Argentine Chaco region, but would be wider up north in the warmer Paraguayan and Bolivian Chaco, and probably occurred year-round in central Brazil (Lehane et al. 1992).

The fact that early spring is an important flight dispersal period of female *T. infestans* is important because following spring house invasion events, the ensuing small populations are unlikely to be detected by the classical timed-manual searches used by vector control personnel to ascertain house infestation status and decide whether the house should be sprayed or not. If viable, these incipient triatomine populations may become detectable after a several months' time lag depending on local conditions and detection methods. An effective vector surveillance system is therefore needed to cope with invading triatomines and low-density triatomine populations.

Acknowledgments

We thank Servicio Metereológico Nacional (Argentina) for providing us with the weather data for La Rioja Airport, Lucía Rodríguez-Planes and Paula M. Gürtler for helpful assistance and comments.

Financial support

R. E. G. acknowledges the support of awards from University of Buenos Aires and Fundación Bunge and Born.

References

Abalos, J. W., and P. Wygodzinsky. 1956. Las vinchucas argentinas. Universidad Nacional de Tucumán, Instituto de Medicina Regional, Publicación No. 705, Folleto de Divulgación No. 7. San Miguel de Tucumán. 37 p.

Abrahan, L. B., D. E. Gorla, and S. S. Catalá. 2011. Dispersal of *Triatoma infestans* and other Triatominae species in the arid Chaco of Argentina - Flying, walking or passive carriage? The importance of walking females. Mem. Inst. Oswaldo Cruz 106: 232-239.

Barbu, C., E. Dumonteil, and S. Gourbiére. 2010. Characterization of the dispersal of non-domiciliated *Triatoma dimidiata* through the selection of spatially explicit models. PLoS Negl. Trop. Dis. 4: e777.

Canale, D. M., M. C. Cecere, R. Chuit, and R. E. Gürtler. 2000. Peridomestic distribution of *Triatoma garciabesi* and *Triatoma guasayana* in north-west Argentina. Med. Vet. Entomol. 14: 383-390.

Carbajal de la Fuente, A. L., S. A. Minoli, C. M. Lopes, F. Noireau, C. R. Lazzari, and M. G. Lorenzo. 2007. Flight dispersal of the Chagas disease vectors *Triatoma brasiliensis* and *Triatoma pseudomaculata* in northeastern Brazil. Acta Trop. 101: 115-119.

Castro, M. C., T. V. Barrett, W. S. Santos, F. Abad-Franch, and J. A. Rafael. 2010. Attraction of Chagas disease vectors (Triatominae) to artificial light sources in the canopy of primary Amazon rainforest. Mem. Inst. Oswaldo Cruz 105: 1061-1064.

Ceballos, L. A., G. M. Vazquez-Prokopec, M. C. Cecere, P. L. Marcet, and R. E. Gürtler. 2005. Feeding rates, nutritional status and flight dispersal potential of peridomestic populations of *Triatoma infestans* in rural northwestern Argentina. Acta Trop. 95: 149-159.

Ceballos, L. A., R. V. Piccinali, P. L. Marcet, G. M. Vazquez-Prokopec, M. V. Cardinal, J. Schachter-Broide, J. P. Dujardin, E. M. Dotson, U. Kitron, and R. E. Gürtler. 2011. Hidden sylvatic foci of the main vector of Chagas disease *Triatoma infestans*: threats to the vector elimination campaign? PLoS Negl. Trop. Dis. 5: e1365.

Cecere, M. C., M. Leporace, M. P. Fernández, J. E. Zárate, C. Moreno, R. E. Gürtler, and M. V. Cardinal. 2016. Host-feeding sources and infection with *Trypanosoma cruzi* of *Triatoma infestans* and *Triatoma eratyrusiformis* (Hemiptera, Reduviidae) from the Calchaqui Valleys in Northwestern Argentina. J. Med. Entomol. 53: 666-673.

Crawford, B. A., and C. M. Kribs-Zaleta. 2013. Vector migration and dispersal rates for sylvatic

Trypanosoma cruzi transmission. Ecol. Complex. 14: 145-156.

Di Iorio, O. R. 2014. A review of the natural history of adult Cetoniinae (Coleoptera: Scarabaeidae) from Argentina and adjacent countries. Zootaxa 3790: 281-318.

Di Iorio, O. R., and P. Turienzo. 2011. A preliminary bibliographic survey of the insects found in poultry houses from the Neotropical Region, with remarks on selected taxa shared with native birds' nests. Zootaxa 2858: 1-60.

Ekkens, D. 1981. Nocturnal flights of *Triatoma* (Hemiptera: Reduviidae) in Sabino Canyon, Arizona. I. Light collections. J. Med. Entomol. 18: 211-227.

Galvão, C., R. U. Carcavallo, D. S. Rocha, and J. Jurberg. 2003. A checklist of the current valid species of the subfamily Triatominae Jeannel, 1919 (Hemiptera, Reduviidae) and their geographical distribution, with nomenclatural and taxonomic notes. Zootaxa 202: 1-36.

Gorla, D. E. and C. J. Schofield (1989) Population dynamics of *Triatoma infestans* under natural climatic conditions in the Argentine Chaco. Med. Vet. Entomol. 3: 179–194.

Gurevitz, J. M., L. A. Ceballos, U. Kitron, and R. E. Gürtler. 2006. Flight initiation of *Triatoma infestans* (Hemiptera: Reduviidae) under natural climatic conditions. J. Med. Entomol. 43: 143-150.

Gurevitz J. M, U. Kitron, and R. E. Gürtler. 2007. Flight muscle dimorphism and heterogeneity in flight initiation of field-collected *Triatoma infestans* (Hemiptera: Reduviidae). J. Med. Entomol. 44: 186-191.

Gurgel-Gonçalves, R., J. B. C. Ferreira, A. F. Rosa, M. E. Bar, and C. Galvão. 2011. Geometric morphometrics and ecological niche modelling for delimitation of near-sibling triatomine species. Med Vet Entomol 25: 84–93.

Gürtler, R. E., M. C. Cecere, D. Canale, M. B. Castañera, R. Chuit, and J. E. Cohen. 1999. Monitoring house reinfestation by vectors of Chagas disease: a comparative trial of detection methods during a four-year follow-up. Acta Trop. 72: 213-234.

Gürtler, R. E., M. C. Cecere, M. P. Fernández, G. M. Vazquez-Prokopec, L. A. Ceballos, J. M. Gurevitz, U. Kitron, and J. E. Cohen. 2014. Key source habitats and potential dispersal of *Triatoma infestans* populations in northwestern Argentina: Implications for vector control. PLoS Negl. Trop. Dis. 8: e3238.

Jácome-Pinilla, D., E. Hincapie-Peñaloza, M. I. Ortiz, J. D. Ramírez, F. Guhl, and J. Molina. 2015. Risks associated with dispersive nocturnal flights of sylvatic Triatominae to artificial lights in a model house in the northeastern plains of Colombia. Parasit. Vectors 19: 600.

Lazzari, C. R. 1992. Circadian organization of locomotion activity in the haematophagous bug *Triatoma infestans*. J. Insect Physiol. 38: 895-903.

Lehane, M. J., P. K. McEwen, C. J. Whitaker, and C. J. Schofield. 1992. The role of temperature and nutritional status in flight initiation by *Triatoma infestans*. Acta Trop. 52: 27-38.

Lent, H., and P. Wygodzinsky. 1979. Revision of the Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas´ disease. Bull. Am. Mus. Nat. Hist. 163: 123-520.

Levy, M. Z., V. R. Quíspe-Machaca, J. L. Ylla-Velasquez, J. M. Richards, B. Rath, K. Borrini-Mayori, L. A. Waller, J. G. Cornejo del Carpio, E. Cordova Benzaquen, F. E. McKenzie, R. A. Wirtz, J. H. Maguire, R. H. Gilman, and C. Bern. 2008. Impregnated netting slows infestation by *Triatoma infestans*. Am. J. Trop. Med. Hyg. 79: 528-534.

Minoli, S. A., and C. R. Lazzari. 2006. Take-off activity and orientation of triatomines (Heteroptera: Reduviidae) in relation to the presence of artificial lights. Acta Trop. 97: 324-330.

Noireau, F., and J. P. Dujardin. 2001. Flight and nutritional status of sylvatic *Triatoma sordida* and *Triatoma guasayana*. Mem. Inst. Oswaldo Cruz 96: 385-389.

Pacheco-Tucuch, F. S., M. J. Ramirez-Sierra, S. Gourbière, and E. Dumonteil. 2012. Public street lights increase house infestation by the Chagas disease vector *Triatoma dimidiata*. PLoS One 7: e36207.

Rodríguez-Planes, L. I., G. M. Vazquez-Prokopec, M. C. Cecere, D. M. Canale, and R. E. Gürtler. 2016. Selective insecticide applications directed against *Triatoma infestans* (Hemiptera: Reduviidae) affected a nontarget secondary vector of Chagas disease, *Triatoma garciabesi*. J. Med. Entomol. 53: 144-151.

Sainz, A. C., L. V. Mauro, E. N. Moriyama, and B. A. García. 2004. Phylogeny of triatomine vectors of *Trypanosoma cruzi* suggested by mitochondrial DNA sequences. Genetica 121: 229-240.

Schofield, C. J., M. J. Lehane, P. McEwen, S. S. Catalá, and D. E. Gorla. 1991. Dispersive flight by *Triatoma sordida*. Trans. R. Soc. Trop. Med. Hyg. 85: 676-678.

Schofield, C. J., M. J. Lehane, P. McEwen, S. S. Catalá, and D. E. Gorla. 1992. Dispersive flight by

Triatoma infestans under natural climatic conditions in Argentina. Med. Vet. Entomol. 6: 51-56.

Sjögren, R. D., and R. E. Ryckman. 1966. Epizootiology of *Trypanosoma cruzi* in southwestern North America Part VIII: nocturnal flights of *Triatoma protracta* (Uhler) as indicated by collections at black light traps. J. Med. Entomol. 3: 81-92.

Stata Corp. 2017. Stata Statistical Software. Release 14.2. College Station: Stata Corporation.

Stroppa, M. M., M. S. Lagunas, C. S. Carriazo, B. A. Garcia, G. Iraola, Y. Panzera, and N. M. Gerez de Burgos. 2013. Differential expression of glycerol-3-phosphate dehydrogenase isoforms in flight muscles of the Chagas disease vector *Triatoma infestans* (Hemiptera, Reduviidae). Am. J. Trop. Med. Hyg. 88: 1146-1151.

Turienzo, P., and O. R. Di Iorio. 2014. Insects in birds' nests from Argentina. *Pseudoseisura lophotes* Reichenbach, 1853 and *Anumbius annumbi* (Vieillot, 1817) (Aves: Furnariidae), hosts of *Triatoma platensis* Neiva, 1913 (Hemiptera: Reduviidae: Triatominae). Zootaxa 3766: 1-82.

Vazquez-Prokopec, G. M., L. A. Ceballos, P. L. Marcet, M. C. Cecere, M. V. Cardinal, U. Kitron, and R. E. Gürtler. 2006. Seasonal variations in active dispersal of natural populations of *Triatoma infestans* in rural north-western Argentina. Med. Vet. Entomol. 20: 273-279.

Vazquez-Prokopec, G. M., M. C. Cecere, U. Kitron, and R. E. Gürtler. 2008. Environmental and demographic factors determining the spatial distribution of *Triatoma guasayana* in peridomestic and semi-sylvatic habitats of rural northwestern Argentina. Med. Vet. Entomol. 22: 273-282.

Vezzani, D., N. J. Schweigmann, S. M. Pietrokowsky, and C. Wisnivesky-Colli. 2001. Characterization of *Triatoma guasayana* biotopes in a hardwood forest of Santiago del Estero, Argentina. Mem. Inst. Oswaldo Cruz 96: 459-466.

Wygodzinsky, P., and J. W. Abalos. 1950. *Triatoma guasayana* Wygodzinsky y Abalos, 1949, con notas sobre especies vecinas (Triatominae, Reduviidae, Hemiptera). An. Inst. Med. Reg. Tucumán 3: 53-73.

Yeo, M., N. Acosta, M. Llewellyn, H. Sánchez, S. Adamson, G. A. Miles, E. López, N. González, J. S. Patterson, M. W. Gaunt, A. R. de Arias, and M. A. Miles. 2005. Origins of Chagas disease: *Didelphis* species are natural hosts of *Trypanosoma cruzi* I and armadillos hosts of *Trypanosoma cruzi* II, including hybrids. Int J Parasitol. 35: 225-233.

Zacharias, C. A., G. B. Pontes, M. G. Lorenzo, and G. Manrique. 2010. Flight initiation by male *Rhodnius prolixus* is promoted by female odors. J. Chem. Ecol. 36: 449-451.

Zeledón, R., J. A. Ugalde, and L. A. Paniagua. 2001. Entomological and ecological aspects of six sylvatic species of triatomines (Hemiptera, Reduviidae) from the collection of the National Biodiversity Institute of Costa Rica, Central America. Mem. Inst. Oswaldo Cruz 96: 757-764.

zu Dohna, H., M. C. Cecere, R. E. Gürtler, U. Kitron, and J. E. Cohen. 2009. Spatial re-establishment dynamics of local populations of vectors of Chagas disease. PLoS Negl. Trop. Dis. 3: e490.

Figure 1. Catch of *Triatoma infestans* (a), *Triatoma guasayana* (b) and *Triatoma eratyrusiformis* (c) adult triatomines at the streetlight posts by sex, month and year at Los Molinos village, 1999-2002 period. Black bars are for males, white bars for females.

Figure 2. Percentage of nights in which at least an adult *Triatoma infestans* (a), *Triatoma guasayana* (b) or *Triatoma eratyrusiformis* (c) was caught at the streetlight posts according to sex and temperature at sunset in Los Molinos village, 1999-2002 period. Solid circles are males, white circles females.

Figure 3. Observed (black dots) and predicted (solid line) percentage of nights in which at least one adult *Triatoma infestans* was caught at the streetlight posts in non-rainy days according to temperature at sunset in Los Molinos village, 1999-2002 period. Dashed lines are the upper and lower bounds of the 95% confidence interval.

Table 1. Overall catch of adult *Triatoma* (Hemiptera: Reduvidae) species in streetlight posts according to species and sex at Los Molinos village, 1999-2002 period

Species	No. (%) of nights with a catch	No. of triatomines collected			% females	Mean (SD) catch per 100 light trap-nights
		Males	Females	Total		
T. guasayana	75 (17.6)	94	28	122	23.0	28.7 (78.8)
T. infestans	60 (14.1)	47	42	89	47.2	20.9 (63.4)
T. eratyrusiformis	51 (12.0)	49	23	72	31.9	16.9 (53.2)
T. garciabesi	5 (1.2)	5	0	5	0	1.2 (10.8)

Table 2. Concurrent catch of both sexes of *T. infestans*, *T. guasayana* and *T. eratyrusiformis* at the same night in Los Molinos village, 1999-2002

Species	Male collected	Female collected		Exact McNemar's χ^2 test with 1 df
		Yes	No	
Triatoma infestans	Yes	11	24	
	No	25	365	*P* = 1.00
Triatoma guasayana	Yes	9	51	
	No	15	350	*P* < 0.0001
Triatoma eratyrusiformis	Yes	10	29	
	No	12	374	*P* = 0.012

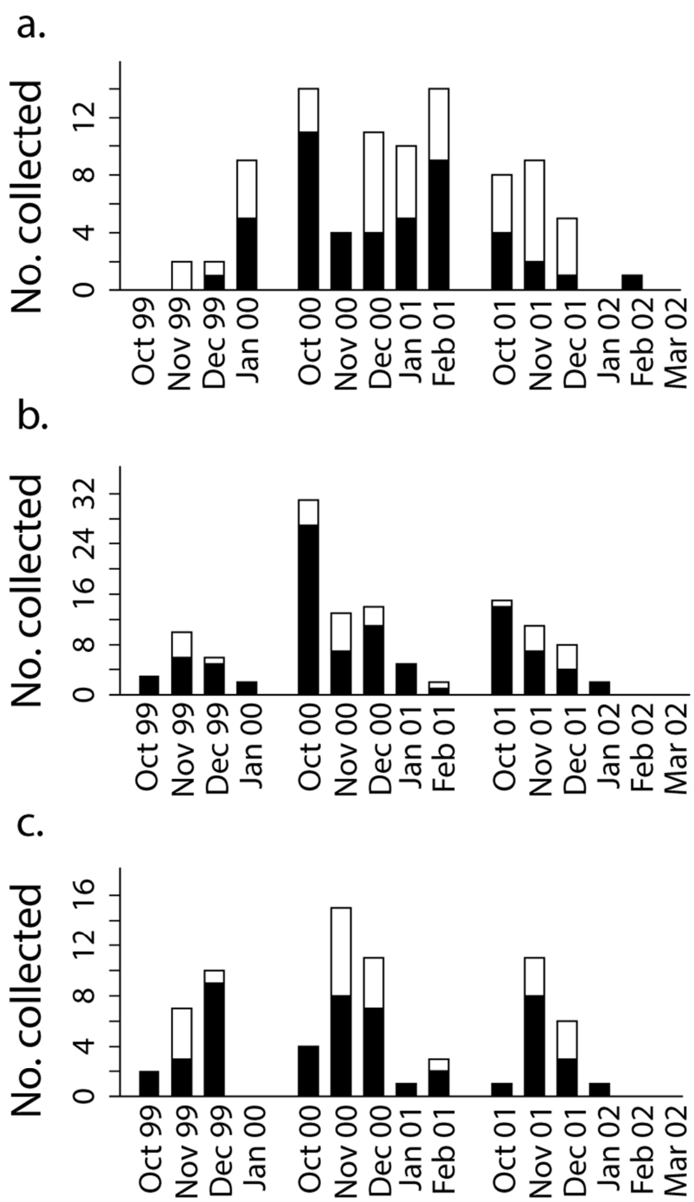

Figure 1. Catch of Triatoma infestans (a), Triatoma guasayana (b) and Triatoma eratyrusiformis (c) adult triatomines at the streetlight posts by sex, month and year at Los Molinos village, 1999-2002 period. Black bars are for males, white bars for females.

73x116mm (300 x 300 DPI)

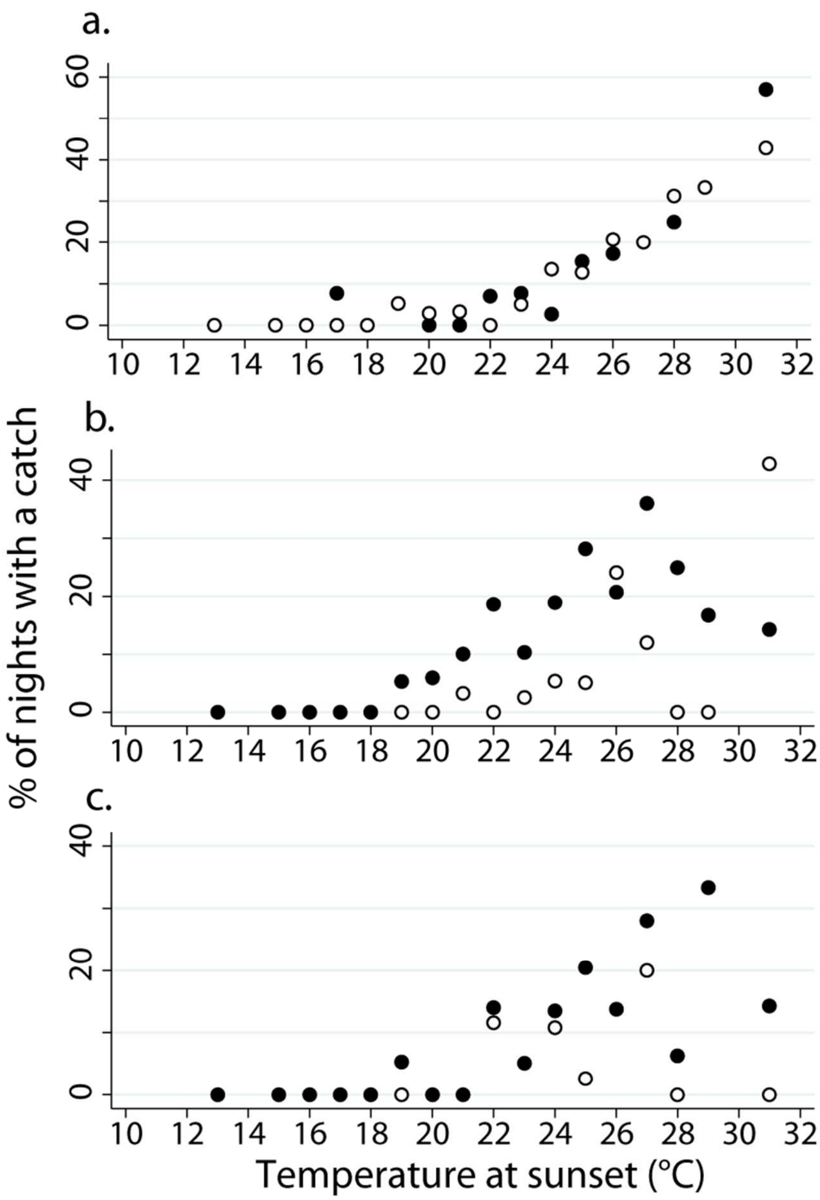

Figure 2. Percentage of nights in which at least an adult Triatoma infestans (a), Triatoma guasayana (b) or Triatoma eratyrusiformis (c) was caught at the streetlight posts according to sex and temperature at sunset in Los Molinos village, 1999-2002 period. Solid circles are males, white circles females.

68x99mm (300 x 300 DPI)

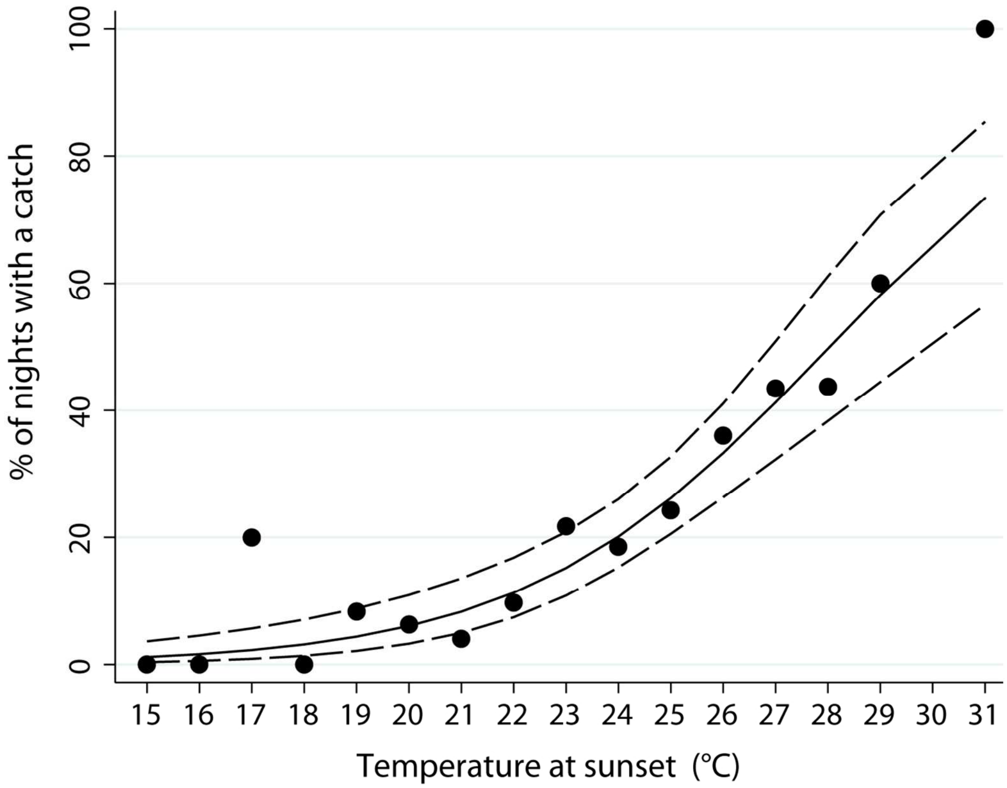

Figure 3. Observed (black dots) and predicted (solid line) percentage of nights in which at least one adult Triatoma infestans was caught at the streetlight posts in non-rainy days according to temperature at sunset in Los Molinos village, 1999-2002 period. Dashed lines are the upper and lower bounds of the 95% confidence interval.

124x97mm (300 x 300 DPI)

Di Iorio and Gürtler.
Seasonal flight dispersal of Chagas disease vectors.

Sampling, Distribution, Dispersal

R. E. Gürtler
Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBA), Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina.
Tel/fax: (5411) 4576-3318
E-mail: gurtler@ege.fcen.uba.ar

Seasonality and temperature-dependent flight dispersal of *Triatoma infestans* and other vectors of Chagas disease in western Argentina

Osvaldo Di Iorio,[1,†] and Ricardo E. Gürtler[2,3]

[1] Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales, Departamento de Biodiversidad y Biología Experimental, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina, and † deceased.

[2] Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales, Departamento de Ecología, Genética y Evolución, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina.

[3] Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad de Buenos Aires. Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBA), Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina.

Abstract

Flight dispersal of Triatominae is affected by climatic conditions and determines the spatiotemporal patterns of house invasion and transmission of *Trypanosoma cruzi* Chagas (Kinetoplastida: Trypanosomatidae). We investigated the detailed time structure and temperature dependencies of flight occurrence of *Triatoma infestans* Klug (Hemiptera: Reduviidae) and other triatomine species in a rural village of western Argentina by taking advantage of the attraction of adult triatomines to artificial light sources. Most of the village's streetlight posts were systematically inspected for triatomines twice between sunset and midnight over 425 nights in the spring-summer seasons of 1999-2002, an unprecedented light-trap sampling effort for any triatomine species. A total of 288 adults were captured, including 122 *Triatoma guasayana* Wygodzinsky and Abalos, 89 *T. infestans*, 72 *Triatoma eratyrusiformis* Del Ponte and 5 *Triatoma garciabesi* Carcavallo et al. Adult sex ratios were balanced in *T. infestans* and strongly male-biased in other species. Nearly all flight-dispersing triatomines were caught when temperatures at sunset were above 20°~~º~~C (range, 16.6-31.7°C), suggesting a putative threshold around 17-18°C. ~~Bug~~Triatomine catches were rare on rainy days. Logistic regression analysis revealed that the proportion of nights in which at least an adult *T. infestans* was caught increased highly significantly with increasing temperature at sunset and was modified by collection month, with greater catches in early spring and no sex differential. This study confirms that spring represents a previously overlooked, important dispersal period of *T. infestans*, and shows large variations among and within Triatominae in their temporal patterns of flight occurrence, abundance and sex ratio.

Key words *Triatoma guasayana*, *Triatoma eratyrusiformis*, *Trypanosoma cruzi*, vector control, temperature, weather

Active dispersal of Triatominae (Hemiptera: Reduviidae) determines the spatial patterns of house invasion and vector infection with *Trypanosoma cruzi* Chagas (Kinetoplastida: Trypanosomatidae), the etiologic agent of Chagas disease (Vazquez-Prokopec et al. 2006, Levy et al. 2008, Barbu et al. 2011). These patterns, derived from environmental conditions combined with nutritional and reproductive status, modify the flight activity of triatomines~~ bugs~~ (Sjögren and Ryckman 1966, Ekkens 1981, Lehane et al. 1992, Schofield et al. 1992, Gurevitz et al. 2006). For example, higher triatomine densities per host would impair the insects' nutritional status (measured by body weight-to-length ratios, W:L), increase the likelihood of flight initiation, and contribute to the regulation of local population density (Lehane et al. 1992, Schofield et al. 1992). Artificial light sources usually attract adult triatomines of many species and favor house invasion (Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2006, Minoli and Lazzari 2006, Carbajal de la Fuente et al. 2007, Castro et al. 2010, Abrahan et al. 2011, Pacheco-Tucuch et al. 2012, Jácome-Pinilla et al. 2015).

Seasonal variations in flight dispersal are fairly common in Triatominae, with flights occurring within the first 2-3 h after sunset in most of the species investigated in the field (Sjögren and Ryckman 1966, Schofield et al. 1992, Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2006). For example, the flight activity of *Triatoma protracta* Uhler and *Triatoma rubida* Neiva peaked in summer in the Arizona desert (Sjögren and Ryckman 1966, Ekkens 1981), whereas flights of *Triatoma dimidiata* Latreille and other species peaked in late spring (dry season) in the forests of Costa Rica (Zeledón et al. 2001). Catches of nine species of Triatominae in a light trap located at canopy level peaked in October-December in the Amazon rainforest (Castro et al. 2010). *Triatoma guasayana* Wygodzinsky and Abalos, a sylvatic or peridomestic species in the dry Chaco ecoregion extending over sections of Argentina, Bolivia and Paraguay, apparently had a distinct flight dispersal window in spring (Gürtler et al. 1999, Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2008, Abrahan et al. 2011, Ceballos et al. 2011), but light-trap efforts at other times a~~we~~re rather sparse.

The flight initiation probabilities of *Triatoma infestans* Klug, the vector responsible for the majority of human infections with *T. cruzi*, significantly decreased with increasing W:L ratios and increased with ambient temperature (Lehane et al. 1992, Schofield et al. 1992, Gurevitz et al. 2006). Light-trap surveys and cage experiments under natural climatic conditions showed that *T. infestans* initiated flight between 22 and 23°C (Vazquez-Prokopec et al. 2006, Gurevitz et al. 2006), whereas in a laboratory setting flight initiation was rare at 18°C and then increased steeply between 20 and 30°C

(Lehane et al. 1992). Whether there is a threshold temperature at which triatomines initiate flight and the functional relationship between flight activity and ambient temperature merit further investigation.

The flight dispersal rate of *T. infestans* was predicted and observed to peak in late summer in the dry Argentine Chaco, when adult triatomines~~bugs~~ also peaked in abundance and reached minimal W:L ratios (Ceballos et al. 2005, Vazquez-Prokopec et al. 2006). However, a detailed analysis of the spatio-temporal patterns of site infestation in this rural area over 10 years (zu Dohna et al. 2009) and a habitat-specific flight index supported that early spring may represent an important dispersal period of *T. infestans* which previous limited light-trap efforts had not detected (Gürtler et al. 2014). Thus, the precise time window at which flight dispersal occurs and potential differences between sexes (Ekkens 1981) remain unknown. This knowledge may be used for modeling house invasion and colonization (e.g. Crawford and Kribs-Zaleta 2013) and has implications for the optimal timing of vector control actions.

A~~s part of a broader investigation on Coleoptera, Di Iorio (2014)~~ systematic~~ally~~ survey~~ed~~ of most of the public streetlights of a small rural village in western Argentina was conducted between sunset and midnight over three consecutive spring-summer seasons (Di Iorio 2014). This unprecedented light-trap sampling effort for triatomines yielded a sizable number of specimens of *T. infestans, T. guasayana, Triatoma eratyrusiformis* Del Ponte and *Triatoma garciabesi* Carcavallo et al. We used this information to investigate whether spring and summer may represent important dispersal periods of *T. infestans* and *T. guasayana*, respectively, and the relationship between flight dispersal, temperature at sunset and triatomine collection month.

Materials and methods

Study area. Field work was conducted at Los Molinos (28° 43' 60'' S, 66° 55' 60'' W), a small rural village with approximately 600 people located at 1,254 m above sea level in an arid zone of La Rioja province (Departamento Castro Barros), western Argentina. The village was situated on low terrain, which determined the virtual absence of strong winds. Most of Los Molinos village comprised abandoned orchards with artificial irrigation and houses with well-plastered brick walls and tin, cement or tiled roofs. Householders usually raised chickens, goats, dogs and pigs. Estates were separated by old fences made with piled rocks. High-pressure mercury-vapor lamps (250 watts, which radiate predominantly in the ultraviolet and visible regions of the optical range) were used for street lighting. Mercury vapor lamps are energetically more efficient than incandescent and most fluorescent lights, with luminous efficacies of 35 to 65 lumens/watt (i.e., a high-intensity, clear white light output). Local residents reported that Chagas vector control programs had not sprayed their houses with insecticide between 1991 and 2002. The local climate was markedly seasonal, with dry (fall-winter) and wet (spring-summer) periods.

Mean monthly temperatures at sunset increased from 19.9°C in October to 23.1-23.6°C between December and February, with substantial variations within the same month over the study period (Di Iorio 2014). Monthly temperatures during the second summer (December 2000-February 2001) were on average 2-3°C higher than in the previous or subsequent years. Monthly minima at sunset ranged from 11.0 to 13.3°C between October and December, and increased to 16.7-17.3°C between January and March throughout the study period. The percentage of days with rainfall was much less frequent in October (mean, 7.5%; range, 0.0-12.9%) or November (10.8%; 9.7-12.9%) than in December (25.8%; 12.9-41.9%), January (36.6%; 32.3-41.8%) and February (25.8%; 19.4-32.3%). The second spring-summer period (2000-2001) was unusually wet relative to the previous and subsequent years, with much greater~~100%~~ herbaceous coverage and ~~peak~~ insect abundance in several areas.

Study design. A longitudinal study was conducted during three consecutive spring-summer seasons (October 1999-January 2000, October 2000-February 2001, and October 2001-March 2002) using a systematic survey design (Di Iorio 2014). Every survey night all 29 mercury-vapor lamps were systematically and sequentially visited twice between sunset and midnight over a total of 425 nights. Each sampling round was organized into five pathways. Because some sections of the village had no alternative access, 14 light posts were inspected twice in each round: one on the way to the end of the street and another on the way back. The ground, vegetation, light poles and other suitable places located within and adjacent to the light cone below each lamp were visually inspected for insects within a 5-15 min sampling period by the senior author. All insects of interest were collected manually, put in killing jars, and later transferred to storage boxes~~cotton beds~~. All collected specimens were subsequently re-examined for an independent corroboration and identified to species following Wygodzinsky and Abalos (1950) and Lent and Wygodzinsky (1979) and nomenclature proposed by Galvão et al. (2003) and Sainz et al. (2004).

Temperatures were recorded exactly at sunset with a digital thermo-hygrometer, except in October 1999 and February 2000, and with a red-colored alcohol thermometer in November and December 1999 for a total of 425 days. These data were compared with those collected by a weather station at La Rioja airport at 500 m of altitude, approximately 200 km from Los Molinos village (Servicio Meteorológico Nacional, unpublished data). Both data sets showed an average difference of 6.8°C at sunset (N = 392 observations) and therefore the airport weather station data were excluded from further consideration.

Data analysis. The database included 425 daily observations on the species-specific occurrence and number of triatomines caught at the streetlights, of which 394 also had records of temperatures at sunset and rainy weather. Maximum-likelihood logistic regression was used to assess the effects of temperature at sunset (a continuous variable) and triatomine collection month (a categorical variable) on the catch of at least one *T. infestans* on a particular night (binary response variable). Triatomine collection month had five levels representing January, February, November and October relative to December, taken as the reference level. This analysis excluded March (when no triatomine~~bug~~ was collected); pooled data over year of collection to avoid small-sample bias, and included 289 observations with complete data on the selected predictors. We verified whether there was any non-linear effect of temperatures at sunset by adding a squared term, which was non-significant for the three species, and run separate regressions for males and females to assess whether model coefficients were significantly different. The goodness of fit of the model was assessed by the Hosmer-Lemeshow test and the area under the Receiver Operating Characteristic (ROC) curve by using 10 quantiles. All analyses were run on Stata 14.2 (StataCorp 2017) using commands *logistic*, *estat gof* and *lroc*. The same analysis was conducted for *T. guasayana* and *T. eratyrusiformis*; catches of *T. garciabesi* were sparse and, therefore, were excluded from detailed analysis. The co-occurrence of male and female adults of a triatomine species on a particular night was tested with a two-sided exact McNemar's test.

Results

A total of 288 specimens of four species of Triatominae were captured at the streetlight posts between 1999 and 2002 (Table 1). Adult triatomines were spotted resting on the substrate or walking within the light cone when they were captured. *Triatoma guasayana* was the most abundant species, representing 42.4% of the total catch, followed by *T. infestans* (30.9%) and *T. eratyrusiformis* (25.0%). Only 5 (1.7%) specimens of *T. garciabesi* were caught. This ranking also held for the average catch per trap-night and the frequency of sample nights in which each species was collected: *T. guasayana* was caught in 75 (17.6%) of 425 sample nights, closely followed by *T. infestans* (14.1%), *T. eratyrusiformis* (12.0%), and *T. garciabesi* (1.2%). Overall adult sex ratios were fairly balanced in *T. infestans* (47.2% of females) and highly skewed toward males in the other species. All specimens were unfed when they were captured as confirmed by visual inspection.

Most of the catches of *T. infestans* comprised only one insect (on 44 occasions); 2, 3, 4 and ≥5 insects were collected during the same night on 9, 3, 2 and 2 occasions, respectively. The frequency distributions of 1, 2, 3, 4 and -5 adult triatomines collected were 50, 15, 4, 1 and 5 for *T. guasayana*, and 37, 9, 3, 2 and 0 for *T. eratyrusiformis*, respectively. Male *T. guasayana* and *T. eratyrusiformis* were caught significantly more often than females of the corresponding species during the same night, but this relationship did not hold for *T. infestans* (Table 2).

Most adults of *T. infestans* were collected over the second spring-summer season (53 of 89, 60%), with peak catches at October and February (Fig. 1A). No catches occurred in October and few in November-December of the first spring-summer season, whereas nearly all catches occurred between October and December of the third season. The overall adult sex ratio displayed substantial variations among months (Fig. 1A). Pooling monthly data over sample years, the female-to-male ratio (female:male) favored males in October (7:15), females in November (9:6) and December (12:6), neither sexone in January (9:10), and favored males again in February (5:10).

Nearly all catches of flight-dispersing triatomines (282 of 288, 97.9%) occurred when temperatures at sunset were above 20º°C (range, 16.6-31.7°C) (Fig. 2A-C). Only in one (3%) of the 92 rainy days recorded throughout the study was an adult triatomine caught at the streetlights. For *T. infestans*, the minimum temperature at sunset at which an adult triatomine was captured was 16.6°C (in October) and the maximum was at 31.7°C (in February) (Fig. 2A). The logit of the proportion of sample nights in which at least an adult *T. infestans* was caught on a non-rainy day (y) increased significantly with temperature at sunset (x_1) and was modified by collection month of insect ($x_{2\text{-}5}$) ($y = 0.4312$ (SE, 0.0685) $x_1 - 0.2617$ (SE, 0.5319) $x_2 - 0.5706$ (SE, 0.4977) $x_3 + 0.2515$ (SE, 0.4947) $x_4 + 1.4126$ (SE, 0.5593) $x_5 - 11.8796$ (SE, 1.7756); $\chi^2 = 63.06$, df = 5, $P < 0.0001$, pseudo $R^2 = 0.216$), where $x_{2\text{-}5}$ represent January, February, November and October, respectively, taking December as the reference level). The temperature-adjusted relative odds of a catch was fourfold greater in October than in December (OR = 4.11, 95% confidence interval, 1.37-12.29). The model displayed a close fit to the data (Hosmer-Lemeshow $\chi^2 = 5.66$, 8 df, $P = 0.685$), with total observations for each quantile ranging from 26 to 33. The area under the ROC curve was 0.815. Model coefficients were not significantly modified by sex of insect. Figure. 3 shows the observed and predicted percentage of nights with a catch (both sexes combined) as a function of temperatures at sunset between 15 and 31°C; only two data points at both extremes deviated significantly from predictions.

For *T. guasayana*, nearly 50% of all specimens were caught during the second season (Fig. 1B). The flight period spanned from October to December, with very few specimens collected from January to February and peak catches in October. The overall sex ratio was consistently male-biased. The minimum and maximum temperatures at which specimens were captured were 19.4 and 30.7°C, respectively (Fig. 2B). The logistic regression model displayed a close fit to the observed proportion of nights in which at least a *T. guasayana* was caught (Hosmer-Lemeshow $\chi^2 = 4.91$, 8 df, $P = 0.767$), and the area under the ROC curve was 0.817 ($y = 0.3661$ (SE, 0.0605) $x_1 - 2.7058$ (SE, 0.8237) $x_2 - 1.3746$ (SE, 0.5198) $x_3 + 0.9211$ (SE, 0.4362) $x_4 + 1.3365$ (SE, 0.5107) $x_5 - 9.8437$ (SE, 1.5477); $\chi^2 = 70.67$, df = 5, $P < 0.0001$, pseudo $R^2 = 0.224$).

For *T. eratyrusiformis*, the flight period also extended from October to December, with peak catches in November-December and very few specimens collected over January and February (Fig. 1C). The overall sex ratio was male-biased throughout the observation period. The minimum and maximum temperatures at which specimens were captured were 19.4 and 29.8°C, respectively (Fig. 2C). As with the other species, the logistic model displayed a close fit to the data (Hosmer-Lemeshow $\chi^2 = 10.46$, 8 df, $P = 0.234$), and the area under the ROC curve was 0.844 ($y = 0.3415$ (SE, 0.0665) $x_1 - 1.9035$ (SE, 0.7123) $x_2 - 3.2070$ (SE, 1.0640) $x_3 + 0.8151$ (SE, 0.4513) x_4 –0.2610 (SE, 0.6110) $x_5 - 9.5031$ (SE, 1.7074); $\chi^2 = 60.49$, df = 5, $P < 0.0001$, pseudo $R^2 = 0.242$).

Discussion

The most important results of our study are related to the seasonal timing of flight dispersal in *T. infestans* and other triatomines, and the close relationship between flight occurrence toward streetlights and temperatures at sunset. Our study documents a large number of dispersing adults of *T. infestans,* which were mainly collected during spring of the second year (the warmest over the 3-yr period), with greater catches in October. This pattern is consistent with the outcomes of a detailed time-series analysis of site occupancy of *T. infestans* over 10 years (zu Dohna et al. 2009), and habitat-specific flight indices based on blood-feeding patterns and other population attributes in the dry Chaco mid-spring (Gürtler et al. 2014). These patterns were not supported by other light-trap surveys with sparse sampling during spring (Vazquez-Prokopec et al. 2006, Abrahan et al. 2011). Taken together, the evidence strongly refutes that flight dispersal of *T. infestans* is rare in spring. We note, however, that weather conditions (including temperatures at sunset) were different in Los Molinos, situated in arid western Argentina at 1,200 m of altitude, than in the dry Chaco region of Santiago del Estero at less than 200 m of elevation. Insect flights at dusk declined steeply during March at the higher altitude of Los Molinos (Di Iorio, unpublished data), unlike in the dry Chaco studies. The spring flight dispersal window is likely to be caused by a chain of events associated with rising spring temperatures and increasing day length after the cold winter months in which triatomines remained metabolically inactive, followed by peak adult emergence (Gorla and Schofield 1980) and the 3- or 4-wk period needed for flight muscle development (Gurevitz et al. 2006). Spring flights are finally made evident under suitable weather conditions.

The occurrence of an apparent spring flight window~~pulse~~ in *T. infestans* does not alter the fact that flight dispersal is more likely to be recorded during the warmer summer months than on the cooler spring months. This statement is contingent upon the weight-to-length (W:L) distribution of triatomine populations, which itself suffers large seasonal and habitat variations (Ceballos et al. 2005). The large inter-annual variability of weather conditions in the Gran Chaco is similar to the large weather variations recorded in Los Molinos over a limited 3-yr period.

Nearly all specimens of the four triatomine species flew at temperatures at sunset above 20~~º~~°C, suggesting a putative threshold for flight dispersal around 17-18°C. This estimate is consistent with experimental evidence showing that the spontaneous locomotory activity of male *T. infestans* would be strongly reduced below 20°C (Lazzari 1992), whereas flight initiation steeply increased from 0% at 15°C, 6.2% at 18°C to 35.2% at 22°C (after pooling data for both sexes and repeat experiments at given temperatures in Table 1 of Lehane et al. 1992). Such thresholds may be related to the activity of the two isoforms of the enzyme glycerol-3-phosphate dehydrogenase, which is crucially linked to flight metabolism in triatomines (Stroppa et al. 2013).

The relative odds of flight occurrence of *T. infestans* toward the streetlights increased highly significantly with increasing temperature at sunset in both sexes. Similarly, flight initiation probabilities increased sigmoidally with increasing temperature at sunset in caged experimental huts under natural climatic conditions, although females displayed consistently higher flight probabilities than males after adjusting for W:L effects (Gurevitz et al. 2006). Both metrics are intimately linked by the same temperature-dependent, binomial process: flight initiation probabilities most likely generated the proportions of nights in which at least one *T. infestans* flew toward the streetlights. In the experimental huts, the asymptote recorded over 25-30°C was in part determined by the large fraction of adult ~~bugs~~triatomines lacking flight muscles (Gurevitz et al. 2007) whereas in the current study there was no asymptote. Here the proportion of females among flight-dispersing adults of *T. infestans* (47.2%) was substantially larger than in light-trap surveys conducted in Santiago del Estero (33.3% of 21 adults) (Vazquez-Prokopec et al. 2006) and in La Rioja (37.5% of 8 adults) (Abrahan et al. 2011). For comparison, the mean percentage of adult females over domestic and peridomestic populations of *T. infestans* averaged 41.7% and varied substantially among ecotopes in the mid-spring of Santiago del Estero (Gürtler et al. 2014).

Our study documents different patterns of flight occurrence, abundance and adult sex ratios among the four species of Triatominae investigated, with large variations among and within years for any one species running in parallel to wide variations in weather conditions. The four triatomine species were most frequently collected at the streetlights during the second (2000-2001) summer period, which was substantially warmer and wetter than the previous or subsequent summers, and only nine (3.1%) of all triatomines collected were caught on a rainy day. In addition, males of *T. guasayana* and *T. eratyrusiformis* occurred significantly more frequently than females of each species, rejecting the hypothesis of independence between sexes. These male-biased patterns may perhaps be explained by pheromone-mediated searches for mates, with an increasing frequency of male fliers in response to female pheromones (Zacharias et al. 2010), and he lower probabilities of flight dispersal of gravid females.

Triatoma guasayana was the species most frequently collected at the streetlights, in agreement with previous light-trap and manual-search surveys in the dry Argentine, Bolivian and Paraguayan Chaco regions (Noireau and Dujardin 2001, Vezzani et al. 2001, Yeo et al. 2005, Vazquez-Prokopec et al. 2008, Ceballos et al. 2011). *Triatoma guasayana* usually occursred in sylvatic conditions under the loose bark of trees or in rocks, fallen trees, dry cacti, bromeliads and logs, and also colonizesd goat or sheep corrals, piled materials, and orchard fences (Canale et al. 2000, Vazquez-Prokopec et al. 2008, Di Iorio and Turienzo 2011). In contrast to female-biased or balanced adult sex ratios (Noireau and Dujardin 2001, Ceballos et al. 2011, Abrahan et al. 2011), the current data show a strong predominance of *T. guasayana* males over seasons and years.

Triatoma eratyrusiformis, a large species found in arid western Argentina, was less abundant and appeared slightly later than *T. guasayana* during spring. The former species iwas sometimes collected in domestic or peridomestic premises (Lent and Wygodzinsky 1979, Di Iorio and Turienzo 2011), and unlike *T. guasayana* and *T. garciabesi*, *T. eratyrusiformis* iwas frequently infected with *T. cruzi* through its association with peridomestic rodents (Cecere et al. 2016).

Triatoma garciabesi was rarely collected at the streetlight posts. This species is frequently found in chicken coops, bird nests and in the rugged bark of *Prosopis* trees where chickens roosted (Canale et al. 2000, Turienzo and Di Iorio, 2014, Rodríguez-Planes et al. 2016). Although *T. garciabesi* outnumbered *T. guasayana* in the peridomestic habitats of Santiago del Estero over time (Canale et al. 2000, Rodríguez-Planes et al. 2016), the former was rarely collected using light traps or by householders as part of routine vector surveillance; none of them colonized human sleeping quarters (Gürtler et al. 1999). Therefore, the marginal light-trap catches of *T. garciabesi* in the study village and elsewhere in La Rioja (Abrahan et al. 2011) may not necessarily imply low abundance in the surrounding habitats. The rare appearance of *T. garciabesi* in light-trap catches and in householders' ~~bug~~triatomine collections is in stark contrast with the frequent flight behavior of *Triatoma sordida* Stål (Schofield et al. 1991) despite the former being a cryptic species within the *sordida* complex (Gurgel-Gonçalvez et al. 2011).

The interpretation of our results is limited by lack of additional information. Data on wind speed may be important for more accurate predictions of flight occurrence because winds greater than 5-6 km/h apparently discourage the initiation of flight in *T. infestans* under natural conditions (Vazquez-Prokopec et al. 2006). In Los Molinos, however, its low-land~~relative~~ location reduced the frequency of strong winds. Adult triatomines most likely flew toward the high-intensity white lights (Minoli and Lazzari 2006), as expected from their unfed status, but some ~~bugs~~ may have walked from nearby sources. The village did not undergo any major disturbance and most likely remained infested at rather similar levels throughout the study period, but local triatomine~~bug~~ abundance and the nutritional status of the putative source ~~bug~~ populations were not investigated. Given the lack of government-sponsored house spraying with insecticide during the previous decade, a reasonable assumption is that *T. infestans* population density fluctuated seasonally around average values. The rest of the triatomine species collected in this study have extensive sylvatic and peridomestic foci and, therefore, were less likely to be affected than *T. infestans*. The current study covered a wide range of climatic conditions over three consecutive spring-summer seasons, but sample periods within any one year did not completely overlap and end-of-summer data (March) were sparse. The extent of the sampling effort did not allow more detailed observations at the level of individual streetlights; local effects were apparent, with few light posts yielding most catches. The systematic search of streetlights provided ample coverage across a well-defined area over several spring-summer seasons, which constitutes an unprecedented light-trap sampling effort of triatomines.

Our current results have implications for improved vector and disease control and modeling of house invasion via flight dispersal: i) adult triatomines of various species attracted to public streetlights are more likely to invade the neighboring houses, leading to spatial clustering of infestation in the vicinities of artificial light sources, as reported for *T. dimidiata* in Yucatan (Pacheco-Tucuch et al. 2012) and *Rhodnius prolixus* Stål in Colombia (Jácome-Pinilla et al. 2015). Here we show the same pattern is applicable to *T. infestans* in a small rural town. Because adult stages typically display the largest prevalence rates of *T. cruzi* infection, such house invasions may ensue human infection risks; ii) analysis of spatiotemporal infestation patterns suggested that *T. infestans* may disperse further than 1.5 km in open fields (zu Dohna et al. 2009), whereas *T. dimidiata* and *R. prolixus* would be attracted by streetlights up to 100 m and by house lights at least for 110 m, respectively (Barbu et al. 2010, Jácome-Pinilla et al. 2015). Therefore, the exact sources of the dispersing ~~bugs~~triatomines may be quite distant to the target streetlights depending on terrain features and vegetation cover; iii) The predicted increase in minimum temperatures fueled by climate change is expected to widen the time window for triatomine flight dispersal, and most likely increase the rates of house invasion; iv) the relationship between temperatures at sunset and flight occurrence combined with local weather information may be used to predict the frequency of house invasion with *T. infestans* at different locations. The time window for flight dispersal was most likely restricted to the October-March period through most of the dry Argentine Chaco region, but would be wider up north in the warmer Paraguayan and Bolivian Chaco, and probably occurred year-round in central Brazil (Lehane et al. 1992).

The fact that early spring is an important flight dispersal period of female *T. infestans* is important because following spring house invasion events, the ensuing small ~~bug~~ populations~~colonies~~ are unlikely to be detected by the classical timed-manual searches used by vector control personnel to ascertain house infestation status and decide whether the house should be sprayed or not. If viable, these incipient ~~bug~~triatomine populations may become detectable after a several months' time lag depending on local conditions and detection methods. An effective vector surveillance system is therefore needed to cope with invading triatomines and low-density~~incipient~~ triatomine populations~~bug colonies~~.

Acknowledgments

We thank Servicio Metereológico Nacional (Argentina) for providing us with the weather data for La

Rioja Airport, Lucía Rodríguez-Planes and Paula M. Gürtler for helpful assistance and comments.

Financial support

R. E. G. acknowledges the support of awards from University of Buenos Aires and Fundación Bunge and Born.

References

Abalos, J. W., and P. Wygodzinsky. 1956. Las vinchucas argentinas. Universidad Nacional de Tucumán, Instituto de Medicina Regional, Publicación No. 705, Folleto de Divulgación No. 7. San Miguel de Tucumán. 37 p.

Abrahan, L. B., D. E. Gorla, and S. S. Catalá. 2011. Dispersal of *Triatoma infestans* and other Triatominae species in the arid Chaco of Argentina - Flying, walking or passive carriage? The importance of walking females. Mem. Inst. Oswaldo Cruz 106: 232-239.

Barbu, C., E. Dumonteil, and S. Gourbiére. 2010. Characterization of the dispersal of non-domiciliated *Triatoma dimidiata* through the selection of spatially explicit models. PLoS Negl. Trop. Dis. 4: e777.

Canale, D. M., M. C. Cecere, R. Chuit, and R. E. Gürtler. 2000. Peridomestic distribution of *Triatoma garciabesi* and *Triatoma guasayana* in north-west Argentina. Med. Vet. Entomol. 14: 383-390.

Carbajal de la Fuente, A. L., S. A. Minoli, C. M. Lopes, F. Noireau, C. R. Lazzari, and M. G. Lorenzo. 2007. Flight dispersal of the Chagas disease vectors *Triatoma brasiliensis* and *Triatoma pseudomaculata* in northeastern Brazil. Acta Trop. 101: 115-119.

Castro, M. C., T. V. Barrett, W. S. Santos, F. Abad-Franch, and J. A. Rafael. 2010. Attraction of Chagas disease vectors (Triatominae) to artificial light sources in the canopy of primary Amazon rainforest. Mem. Inst. Oswaldo Cruz 105: 1061-1064.

Ceballos, L. A., G. M. Vazquez-Prokopec, M. C. Cecere, P. L. Marcet, and R. E. Gürtler. 2005. Feeding rates, nutritional status and flight dispersal potential of peridomestic populations of *Triatoma infestans* in rural northwestern Argentina. Acta Trop. 95: 149-159.

Ceballos, L. A., R. V. Piccinali, P. L. Marcet, G. M. Vazquez-Prokopec, M. V. Cardinal, J. Schachter-Broide, J. P. Dujardin, E. M. Dotson, U. Kitron, and R. E. Gürtler. 2011. Hidden sylvatic foci of the main vector of Chagas disease *Triatoma infestans*: threats to the vector elimination campaign? PLoS Negl. Trop. Dis. 5: e1365.

Cecere, M. C., M. Leporace, M. P. Fernández, J. E. Zárate, C. Moreno, R. E. Gürtler, and M. V. Cardinal. 2016. Host-feeding sources and infection with *Trypanosoma cruzi* of *Triatoma infestans* and *Triatoma eratyrusiformis* (Hemiptera, Reduviidae) from the Calchaqui Valleys in Northwestern Argentina. J. Med. Entomol. 53: 666-673.

Crawford, B. A., and C. M. Kribs-Zaleta. 2013. Vector migration and dispersal rates for sylvatic

Trypanosoma cruzi transmission. Ecol. Complex. 14: 145-156.

Di Iorio, O. R. 2014. A review of the natural history of adult Cetoniinae (Coleoptera: Scarabaeidae) from Argentina and adjacent countries. Zootaxa 3790: 281-318.

Di Iorio, O. R., and P. Turienzo. 2011. A preliminary bibliographic survey of the insects found in poultry houses from the Neotropical Region, with remarks on selected taxa shared with native birds' nests. Zootaxa 2858: 1-60.

Ekkens, D. 1981. Nocturnal flights of *Triatoma* (Hemiptera: Reduviidae) in Sabino Canyon, Arizona. I. Light collections. J. Med. Entomol. 18: 211-227.

Galvão, C., R. U. Carcavallo, D. S. Rocha, and J. Jurberg. 2003. A checklist of the current valid species of the subfamily Triatominae Jeannel, 1919 (Hemiptera, Reduviidae) and their geographical distribution, with nomenclatural and taxonomic notes. Zootaxa 202: 1-36.

Gorla, D. E. and C. J. Schofield (1989) Population dynamics of *Triatoma infestans* under natural climatic conditions in the Argentine Chaco. Med. Vet. Entomol. 3: 179–194.

Gurevitz, J. M., L. A. Ceballos, U. Kitron, and R. E. Gürtler. 2006. Flight initiation of *Triatoma infestans* (Hemiptera: Reduviidae) under natural climatic conditions. J. Med. Entomol. 43: 143-150.

Gurevitz J. M, U. Kitron, and R. E. Gürtler. 2007. Flight muscle dimorphism and heterogeneity in flight initiation of field-collected *Triatoma infestans* (Hemiptera: Reduviidae). J. Med. Entomol. 44: 186-191.

Gurgel-Gonçalves, R., J. B. C. Ferreira, A. F. Rosa, M. E. Bar, and C. Galvão. 2011. Geometric morphometrics and ecological niche modelling for delimitation of near-sibling triatomine species. Med Vet Entomol 25: 84–93.

Gürtler, R. E., M. C. Cecere, D. Canale, M. B. Castañera, R. Chuit, and J. E. Cohen. 1999. Monitoring house reinfestation by vectors of Chagas disease: a comparative trial of detection methods during a four-year follow-up. Acta Trop. 72: 213-234.

Gürtler, R. E., M. C. Cecere, M. P. Fernández, G. M. Vazquez-Prokopec, L. A. Ceballos, J. M. Gurevitz, U. Kitron, and J. E. Cohen. 2014. Key source habitats and potential dispersal of *Triatoma infestans* populations in northwestern Argentina: Implications for vector control. PLoS Negl. Trop. Dis. 8: e3238.

Jácome-Pinilla, D., E. Hincapie-Peñaloza, M. I. Ortiz, J. D. Ramírez, F. Guhl, and J. Molina. 2015. Risks associated with dispersive nocturnal flights of sylvatic Triatominae to artificial lights in a model house in the northeastern plains of Colombia. Parasit. Vectors 19: 600.

Lazzari, C. R. 1992. Circadian organization of locomotion activity in the haematophagous bug *Triatoma infestans*. J. Insect Physiol. 38: 895-903.

Lehane, M. J., P. K. McEwen, C. J. Whitaker, and C. J. Schofield. 1992. The role of temperature and nutritional status in flight initiation by *Triatoma infestans*. Acta Trop. 52: 27-38.

Lent, H., and P. Wygodzinsky. 1979. Revision of the Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas´ disease. Bull. Am. Mus. Nat. Hist. 163: 123-520.

Levy, M. Z., V. R. Quíspe-Machaca, J. L. Ylla-Velasquez, J. M. Richards, B. Rath, K. Borrini-Mayori, L. A. Waller, J. G. Cornejo del Carpio, E. Cordova Benzaquen, F. E. McKenzie, R. A. Wirtz, J. H. Maguire, R. H. Gilman, and C. Bern. 2008. Impregnated netting slows infestation by *Triatoma infestans*. Am. J. Trop. Med. Hyg. 79: 528-534.

Minoli, S. A., and C. R. Lazzari. 2006. Take-off activity and orientation of triatomines (Heteroptera: Reduviidae) in relation to the presence of artificial lights. Acta Trop. 97: 324-330.

Noireau, F., and J. P. Dujardin. 2001. Flight and nutritional status of sylvatic *Triatoma sordida* and *Triatoma guasayana*. Mem. Inst. Oswaldo Cruz 96: 385-389.

Pacheco-Tucuch, F. S., M. J. Ramirez-Sierra, S. Gourbière, and E. Dumonteil. 2012. Public street lights increase house infestation by the Chagas disease vector *Triatoma dimidiata*. PLoS One 7: e36207.

Rodríguez-Planes, L. I., G. M. Vazquez-Prokopec, M. C. Cecere, D. M. Canale, and R. E. Gürtler. 2016. Selective insecticide applications directed against *Triatoma infestans* (Hemiptera: Reduviidae) affected a nontarget secondary vector of Chagas disease, *Triatoma garciabesi*. J. Med. Entomol. 53: 144-151.

Sainz, A. C., L. V. Mauro, E. N. Moriyama, and B. A. García. 2004. Phylogeny of triatomine vectors of *Trypanosoma cruzi* suggested by mitochondrial DNA sequences. Genetica 121: 229-240.

Schofield, C. J., M. J. Lehane, P. McEwen, S. S. Catalá, and D. E. Gorla. 1991. Dispersive flight by *Triatoma sordida*. Trans. R. Soc. Trop. Med. Hyg. 85: 676-678.

Schofield, C. J., M. J. Lehane, P. McEwen, S. S. Catalá, and D. E. Gorla. 1992. Dispersive flight by

Triatoma infestans under natural climatic conditions in Argentina. Med. Vet. Entomol. 6: 51-56.

Sjögren, R. D., and R. E. Ryckman. 1966. Epizootiology of *Trypanosoma cruzi* in southwestern North America Part VIII: nocturnal flights of *Triatoma protracta* (Uhler) as indicated by collections at black light traps. J. Med. Entomol. 3: 81-92.

Stata Corp. 2017. Stata Statistical Software. Release 14.2. College Station: Stata Corporation.

Stroppa, M. M., M. S. Lagunas, C. S. Carriazo, B. A. Garcia, G. Iraola, Y. Panzera, and N. M. Gerez de Burgos. 2013. Differential expression of glycerol-3-phosphate dehydrogenase isoforms in flight muscles of the Chagas disease vector *Triatoma infestans* (Hemiptera, Reduviidae). Am. J. Trop. Med. Hyg. 88: 1146-1151.

Turienzo, P., and O. R. Di Iorio. 2014. Insects in birds' nests from Argentina. *Pseudoseisura lophotes* Reichenbach, 1853 and *Anumbius annumbi* (Vieillot, 1817) (Aves: Furnariidae), hosts of *Triatoma platensis* Neiva, 1913 (Hemiptera: Reduviidae: Triatominae). Zootaxa 3766: 1-82.

Vazquez-Prokopec, G. M., L. A. Ceballos, P. L. Marcet, M. C. Cecere, M. V. Cardinal, U. Kitron, and R. E. Gürtler. 2006. Seasonal variations in active dispersal of natural populations of *Triatoma infestans* in rural north-western Argentina. Med. Vet. Entomol. 20: 273-279.

Vazquez-Prokopec, G. M., M. C. Cecere, U. Kitron, and R. E. Gürtler. 2008. Environmental and demographic factors determining the spatial distribution of *Triatoma guasayana* in peridomestic and semi-sylvatic habitats of rural northwestern Argentina. Med. Vet. Entomol. 22: 273-282.

Vezzani, D., N. J. Schweigmann, S. M. Pietrokowsky, and C. Wisnivesky-Colli. 2001. Characterization of *Triatoma guasayana* biotopes in a hardwood forest of Santiago del Estero, Argentina. Mem. Inst. Oswaldo Cruz 96: 459-466.

Wygodzinsky, P., and J. W. Abalos. 1950. *Triatoma guasayana* Wygodzinsky y Abalos, 1949, con notas sobre especies vecinas (Triatominae, Reduviidae, Hemiptera). An. Inst. Med. Reg. Tucumán 3: 53-73.

Yeo, M., N. Acosta, M. Llewellyn, H. Sánchez, S. Adamson, G. A. Miles, E. López, N. González, J. S. Patterson, M. W. Gaunt, A. R. de Arias, and M. A. Miles. 2005. Origins of Chagas disease: *Didelphis* species are natural hosts of *Trypanosoma cruzi* I and armadillos hosts of *Trypanosoma cruzi* II, including hybrids. Int J Parasitol. 35: 225-233.

Zacharias, C. A., G. B. Pontes, M. G. Lorenzo, and G. Manrique. 2010. Flight initiation by male *Rhodnius prolixus* is promoted by female odors. J. Chem. Ecol. 36: 449-451.

Zeledón, R., J. A. Ugalde, and L. A. Paniagua. 2001. Entomological and ecological aspects of six sylvatic species of triatomines (Hemiptera, Reduviidae) from the collection of the National Biodiversity Institute of Costa Rica, Central America. Mem. Inst. Oswaldo Cruz 96: 757-764.

zu Dohna, H., M. C. Cecere, R. E. Gürtler, U. Kitron, and J. E. Cohen. 2009. Spatial re-establishment dynamics of local populations of vectors of Chagas disease. PLoS Negl. Trop. Dis. 3: e490.

~~**Table 1.** Overall catch of adult *Triatoma* (Hemiptera: Reduvidae) species in streetlight posts according to species and sex at Los Molinos village, 1999-2002~~

Species	No. (%) of nights with a catch	No. of bugstriatomines collected			% females	Mean (SD) catch per 100 light trap-nights
		Males	Females	Total		
T. guasayana	75 (17.6)	94	28	122	23.0	28.7 (78.8)
T. infestans	60 (14.1)	47	42	89	47.2	20.9 (63.4)
T. eratyrusiformis	51 (12.0)	49	23	72	31.9	16.9 (53.2)
T. garciabesi	5 (1.2)	5	0	5	0	1.2 (10.8)

Formatted: Justified

Formatted: Justified

Formatted: Justified

Formatted: Justified

Formatted: Justified

Formatted: Justified

Formatted: Justified

Table 2. Concurrent catch of both sexes of *Triatoma. infestans*, *Triatoma. guasayana* and *Triatoma. eratyrusiformis* at the same night in Los Molinos village, 1999-2002

Species	Male collected	Female collected		Exact McNemar's χ^2 test with 1 df
		Yes	No	
Triatoma infestans	Yes	11	24	
	No	25	365	*P* = 1.00
Triatoma guasayana	Yes	9	51	
	No	15	350	*P* < 0.0001
Triatoma eratyrusiformis	Yes	10	29	
	No	12	374	*P* = 0.012

Formatted: Justified

Formatted: Justified

Formatted: Justified

Formatted: Justified, Tab stops: 0.4", Left + Not at 0"

Formatted: Justified

Formatted: Justified, Tab stops: 0.4", Left + Not at 0"

Formatted: Justified

Formatted: Justified, Tab stops: 0.4", Left + Not at 0"

Formatted: Justified

Formatted: Justified, Tab stops: 0.4", Left + Not at 0"

Formatted: Justified

Formatted: Justified, Tab stops: 0.4", Left + Not at 0"

Figure 1. Catch of *Triatoma infestans* (a), *Triatoma guasayana* (b) and *Triatoma eratyrusiformis* (c) adult triatomines at the streetlight posts by sex, month and year at Los Molinos village, 1999-2002 period. Black bars are for males, white bars for females.

Figure 2. Percentage of nights in which at least an adult *Triatoma infestans* (a~~A~~), *Triatoma guasayana* (b~~B~~) or *Triatoma eratyrusiformis* (c~~C~~) was caught at the streetlight posts according to sex and temperature at sunset in Los Molinos village, 1999-2002 period. Solid circles are males, white circles females.

Figure 3. Observed (black dots) and predicted (solid line) percentage of nights in which at least one adult *Triatoma~~.~~ infestans* was caught at the streetlight posts in non-rainy days according to temperature at sunset in Los Molinos village, 1999-2002 period. Dashed lines are the upper and lower bounds of the 95% confidence interval.