

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

Journal:	<i>Journal of Medical Entomology</i>
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 (IEGEB),
Please choose a section from the list:	Sampling, Distribution, Dispersal
Field Keywords:	Medical Entomology, Trypanosomiasis, Chagas Disease, Vector Ecology, Surveillance
Organism Keywords:	Triatominae

SCHOLARONE™
Manuscripts

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

4

5 Sampling, Distribution, Dispersal

6

7

8

9

10

11

12

13

14

15

16

17 R. E. Gürtler

18 Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas.

19 Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEB), Facultad de

20 Ciencias Exactas y Naturales, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina.

21 Tel/fax: (5411) 4576-3318

22 E-mail: gurtler@ege.fcen.uba.ar

23

24

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

27

28 Osvaldo Di Iorio,^{1,†} and Ricardo E. Gürtler^{2,3}

29

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

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

35 ³ Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad de Buenos Aires. Instituto de
36 Ecología, Genética y Evolución de Buenos Aires (IEGEB), Ciudad Universitaria, C1428EHA,
37 Buenos Aires, Argentina.

38

39 **Abstract**

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

58

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

61

62 Active dispersal of Triatominae (Hemiptera: Reduviidae) determines the spatial patterns of house
63 invasion and vector infection with *Trypanosoma cruzi* Chagas (Kinetoplastida: Trypanosomatidae), the
64 etiologic agent of Chagas disease (Vazquez-Prokopec et al. 2006, Levy et al. 2008, Barbu et al. 2011).
65 These patterns, derived from environmental conditions combined with nutritional and reproductive status,
66 modify the flight activity of triatomines (Sjögren and Ryckman 1966, Ekkens 1981, Lehane et al. 1992,
67 Schofield et al. 1992, Gurevitz et al. 2006). For example, higher triatomine densities per host would
68 impair the insects' nutritional status (measured by body weight-to-length ratios, W:L), increase the
69 likelihood of flight initiation, and contribute to the regulation of local population density (Lehane et al.
70 1992, Schofield et al. 1992). Artificial light sources usually attract adult triatomines of many species and
71 favor house invasion (Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2006, Minoli and Lazzari
72 2006, Carbajal de la Fuente et al. 2007, Castro et al. 2010, Abrahan et al. 2011, Pacheco-Tucuch et al.
73 2012, Jácome-Pinilla et al. 2015).

74 Seasonal variations in flight dispersal are fairly common in Triatominae, with flights occurring
75 within the first 2-3 h after sunset in most of the species investigated in the field (Sjögren and Ryckman
76 1966, Schofield et al. 1992, Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2006). For example,
77 the flight activity of *Triatoma protracta* Uhler and *Triatoma rubida* Neiva peaked in summer in the
78 Arizona desert (Sjögren and Ryckman 1966, Ekkens 1981), whereas flights of *Triatoma dimidiata*
79 Latreille and other species peaked in late spring (dry season) in the forests of Costa Rica (Zeledón et al.
80 2001). Catches of nine species of Triatominae in a light trap located at canopy level peaked in October-
81 December in the Amazon rainforest (Castro et al. 2010). *Triatoma guasayana* Wygodzinsky and
82 Abalos, a sylvatic or peridomestic species in the dry Chaco ecoregion extending over sections of
83 Argentina, Bolivia and Paraguay, apparently had a distinct flight dispersal window in spring (Gürtler et
84 al. 1999, Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2008, Abrahan et al. 2011, Ceballos et
85 al. 2011), but light-trap efforts at other times are rather sparse.

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

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

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

103 Asystematic survey of most of the public streetlights of a small rural village in western Argentina
104 was conducted between sunset and midnight over three consecutive spring-summer seasons (Di Iorio
105 2014). This unprecedented light-trap sampling effort for triatomines yielded a sizable number of
106 specimens of *T. infestans*, *T. guasayana*, *Triatoma eratyrusiformis* Del Ponte and *Triatoma garciabesi*
107 Carcavallo et al. We used this information to investigate whether spring and summer may represent
108 important dispersal periods of *T. infestans* and *T. guasayana*, respectively, and the relationship between
109 flight dispersal, temperature at sunset and triatomine collection month.

111 **Materials and methods**

112 **Study area.** Field work was conducted at Los Molinos (28° 43' 60'' S, 66° 55' 60'' W), a small rural
113 village with approximately 600 people located at 1,254 m above sea level in an arid zone of La Rioja
114 province (Departamento Castro Barros), western Argentina. The village was situated on low terrain,
115 which determined the virtual absence of strong winds. Most of Los Molinos village comprised abandoned
116 orchards with artificial irrigation and houses with well-plastered brick walls and tin, cement or tiled roofs.
117 Householders usually raised chickens, goats, dogs and pigs. Estates were separated by old fences made
118 with piled rocks. High-pressure mercury-vapor lamps (250 watts, which radiate predominantly in the
119 ultraviolet and visible regions of the optical range) were used for street lighting. Mercury vapor lamps are
120 energetically more efficient than incandescent and most fluorescent lights, with luminous efficacies of 35
121 to 65 lumens/watt (i.e., a high-intensity, clear white light output). Local residents reported that Chagas

122 vector control programs had not sprayed their houses with insecticide between 1991 and 2002. The
123 local climate was markedly seasonal, with dry (fall-winter) and wet (spring-summer) periods.

124 Mean monthly temperatures at sunset increased from 19.9°C in October to 23.1-23.6°C between
125 December and February, with substantial variations within the same month over the study period (Di Iorio
126 2014). Monthly temperatures during the second summer (December 2000-February 2001) were on
127 average 2-3°C higher than in the previous or subsequent years. Monthly minima at sunset ranged from
128 11.0 to 13.3°C between October and December, and increased to 16.7-17.3°C between January and
129 March throughout the study period. The percentage of days with rainfall was much less frequent in
130 October (mean, 7.5%; range, 0.0-12.9%) or November (10.8%; 9.7-12.9%) than in December (25.8%;
131 12.9-41.9%), January (36.6%; 32.3-41.8%) and February (25.8%; 19.4-32.3%). The second spring-
132 summer period (2000-2001) was unusually wet relative to the previous and subsequent years, with much
133 greater herbaceous coverage and insect abundance in several areas.

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

146 Temperatures were recorded exactly at sunset with a digital thermo-hygrometer, except in October
147 1999 and February 2000, and with a red-colored alcohol thermometer in November and December 1999
148 for a total of 425 days. These data were compared with those collected by a weather station at La Rioja
149 airport at 500 m of altitude, approximately 200 km from Los Molinos village (Servicio Meteorológico
150 Nacional, unpublished data). Both data sets showed an average difference of 6.8°C at sunset (N = 392
151 observations) and therefore the airport weather station data were excluded from further consideration.

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

169

170 **Results**

171 A total of 288 specimens of four species of Triatominae were captured at the streetlight posts between
172 1999 and 2002 (Table 1). Adult triatomines were spotted resting on the substrate or walking within the
173 light cone when they were captured. *Triatoma guasayana* was the most abundant species, representing
174 42.4% of the total catch, followed by *T. infestans* (30.9%) and *T. eratyrisiformis* (25.0%). Only 5 (1.7%)
175 specimens of *T. garciabesi* were caught. This ranking also held for the average catch per trap-night and
176 the frequency of sample nights in which each species was collected: *T. guasayana* was caught in 75
177 (17.6%) of 425 sample nights, closely followed by *T. infestans* (14.1%), *T. eratyrisiformis* (12.0%), and
178 *T. garciabesi* (1.2%). Overall adult sex ratios were fairly balanced in *T. infestans* (47.2% of females) and
179 highly skewed toward males in the other species. All specimens were unfed when they were captured as
180 confirmed by visual inspection.

181 Most of the catches of *T. infestans* comprised only one insect (on 44 occasions); 2, 3, 4 and ≥ 5

182 insects were collected during the same night on 9, 3, 2 and 2 occasions, respectively. The frequency
183 distributions of 1, 2, 3, 4 and 5 adult triatomines collected were 50, 15, 4, 1 and 5 for *T. guasayana*, and
184 37, 9, 3, 2 and 0 for *T. eratyrisiformis*, respectively. Male *T. guasayana* and *T. eratyrisiformis* were
185 caught significantly more often than females of the corresponding species during the same night, but this
186 relationship did not hold for *T. infestans* (Table 2).

187 Most adults of *T. infestans* were collected over the second spring-summer season (53 of 89, 60%),
188 with peak catches at October and February (Fig. 1A). No catches occurred in October and few in
189 November-December of the first spring-summer season, whereas nearly all catches occurred between
190 October and December of the third season. The overall adult sex ratio displayed substantial variations
191 among months (Fig. 1A). Pooling monthly data over sample years, the female-to-male ratio (female:male)
192 favored males in October (7:15), females in November (9:6) and December (12:6), neither sex in January
193 (9:10), and favored males again in February (5:10).

194 Nearly all catches of flight-dispersing triatomines (282 of 288, 97.9%) occurred when
195 temperatures at sunset were above 20°C (range, 16.6-31.7°C) (Fig. 2A-C). Only in one (3%) of the 92
196 rainy days recorded throughout the study was an adult triatomine caught at the streetlights. For *T.*
197 *infestans*, the minimum temperature at sunset at which an adult triatomine was captured was 16.6°C (in
198 October) and the maximum was at 31.7°C (in February) (Fig. 2A). The logit of the proportion of sample
199 nights in which at least an adult *T. infestans* was caught on a non-rainy day (y) increased significantly with
200 temperature at sunset (x_1) and was modified by collection month of insect (x_{2-5}) ($y = 0.4312$ (SE, 0.0685)
201 $x_1 - 0.2617$ (SE, 0.5319) $x_2 - 0.5706$ (SE, 0.4977) $x_3 + 0.2515$ (SE, 0.4947) $x_4 + 1.4126$ (SE, 0.5593) $x_5 -$
202 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,
203 February, November and October, respectively, taking December as the reference level. The temperature-
204 adjusted relative odds of a catch was fourfold greater in October than in December (OR = 4.11, 95%
205 confidence interval, 1.37-12.29). The model displayed a close fit to the data (Hosmer-Lemeshow $\chi^2 =$
206 5.66 , 8 df , $P = 0.685$), with total observations for each quantile ranging from 26 to 33. The area under the
207 ROC curve was 0.815. Model coefficients were not significantly modified by sex of insect. Figure 3
208 shows the observed and predicted percentage of nights with a catch (both sexes combined) as a function of
209 temperatures at sunset between 15 and 31°C; only two data points at both extremes deviated significantly
210 from predictions.

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

212 The flight period spanned from October to December, with very few specimens collected from January to
213 February and peak catches in October. The overall sex ratio was consistently male-biased. The minimum
214 and maximum temperatures at which specimens were captured were 19.4 and 30.7°C, respectively (Fig.
215 2B). The logistic regression model displayed a close fit to the observed proportion of nights in which at
216 least a *T. guasayana* was caught (Hosmer-Lemeshow $\chi^2 = 4.91$, 8 df, $P = 0.767$), and the area under the
217 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) $x_3 +$
218 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, $P < 0.0001$,
219 pseudo $R^2 = 0.224$).

220 For *T. eratyrisiformis*, the flight period also extended from October to December, with peak
221 catches in November-December and very few specimens collected over January and February (Fig. 1C).
222 The overall sex ratio was male-biased throughout the observation period. The minimum and maximum
223 temperatures at which specimens were captured were 19.4 and 29.8°C, respectively (Fig. 2C). As with the
224 other species, the logistic model displayed a close fit to the data (Hosmer-Lemeshow $\chi^2 = 10.46$, 8 df, $P =$
225 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)
226 $x_2 - 3.2070$ (SE, 1.0640) $x_3 + 0.8151$ (SE, 0.4513) $x_4 - 0.2610$ (SE, 0.6110) $x_5 - 9.5031$ (SE, 1.7074); χ^2
227 $= 60.49$, df = 5, $P < 0.0001$, pseudo $R^2 = 0.242$).

228

229 Discussion

230 The most important results of our study are related to the seasonal timing of flight dispersal in *T. infestans*
231 and other triatomines, and the close relationship between flight occurrence toward streetlights and
232 temperatures at sunset. Our study documents a large number of dispersing adults of *T. infestans*, which
233 were mainly collected during spring of the second year (the warmest over the 3-yr period), with greater
234 catches in October. This pattern is consistent with the outcomes of a detailed time-series analysis of site
235 occupancy of *T. infestans* over 10 years (zu Dohna et al. 2009), and habitat-specific flight indices based on
236 blood-feeding patterns and other population attributes in the dry Chaco mid-spring (Gürtler et al. 2014).
237 These patterns were not supported by other light-trap surveys with sparse sampling during spring
238 (Vazquez-Prokopec et al. 2006, Abrahan et al. 2011). Taken together, the evidence strongly refutes that
239 flight dispersal of *T. infestans* is rare in spring. We note, however, that weather conditions (including
240 temperatures at sunset) were different in Los Molinos, situated in arid western Argentina at 1,200 m of
241 altitude, than in the dry Chaco region of Santiago del Estero at less than 200 m of elevation. Insect flights

242 at dusk declined steeply during March at the higher altitude of Los Molinos (Di Iorio, unpublished data),
243 unlike in the dry Chaco studies. The spring flight dispersal window is likely to be caused by a chain of
244 events associated with rising spring temperatures and increasing day length after the cold winter months
245 in which triatomines remained metabolically inactive, followed by peak adult emergence (Gorla and
246 Schofield 1980) and the 3- or 4-wk period needed for flight muscle development (Gurevitz et al. 2006).
247 Spring flights are finally made evident under suitable weather conditions.

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

254 Nearly all specimens of the four triatomine species flew at temperatures at sunset above 20°C,
255 suggesting a putative threshold for flight dispersal around 17-18°C. This estimate is consistent with
256 experimental evidence showing that the spontaneous locomotory activity of male *T. infestans* would be
257 strongly reduced below 20°C (Lazzari 1992), whereas flight initiation steeply increased from 0% at 15°C,
258 6.2% at 18°C to 35.2% at 22°C (after pooling data for both sexes and repeat experiments at given
259 temperatures in Table 1 of Lehane et al. 1992). Such thresholds may be related to the activity of the two
260 isoforms of the enzyme glycerol-3-phosphate dehydrogenase, which is crucially linked to flight
261 metabolism in triatomines (Stroppa et al. 2013).

262 The relative odds of flight occurrence of *T. infestans* toward the streetlights increased highly
263 significantly with increasing temperature at sunset in both sexes. Similarly, flight initiation probabilities
264 increased sigmoidally with increasing temperature at sunset in caged experimental huts under natural
265 climatic conditions, although females displayed consistently higher flight probabilities than males after
266 adjusting for W:L effects (Gurevitz et al. 2006). Both metrics are intimately linked by the same
267 temperature-dependent, binomial process: flight initiation probabilities most likely generated the
268 proportions of nights in which at least one *T. infestans* flew toward the streetlights. In the experimental
269 huts, the asymptote recorded over 25-30°C was in part determined by the large fraction of adult
270 triatomines lacking flight muscles (Gurevitz et al. 2007) whereas in the current study there was no
271 asymptote. Here the proportion of females among flight-dispersing adults of *T. infestans* (47.2%) was

272 substantially larger than in light-trap surveys conducted in Santiago del Estero (33.3% of 21 adults)
273 (Vazquez-Prokopec et al. 2006) and in La Rioja (37.5% of 8 adults) (Abraham et al. 2011). For
274 comparison, the mean percentage of adult females over domestic and peridomestic populations of *T.*
275 *infestans* averaged 41.7% and varied substantially among ecotopes in the mid-spring of Santiago del
276 Estero (Gürtler et al. 2014).

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

287 *Triatoma guasayana* was the species most frequently collected at the streetlights, in agreement
288 with previous light-trap and manual-search surveys in the dry Argentine, Bolivian and Paraguayan Chaco
289 regions (Noireau and Dujardin 2001, Vezzani et al. 2001, Yeo et al. 2005, Vazquez-Prokopec et al. 2008,
290 Ceballos et al. 2011). *Triatoma guasayana* usually occurs in sylvatic conditions under the loose bark of
291 trees or in rocks, fallen trees, dry cacti, bromeliads and logs, and also colonizes goat or sheep corrals, piled
292 materials, and orchard fences (Canale et al. 2000, Vazquez-Prokopec et al. 2008, Di Iorio and Turienzo
293 2011). In contrast to female-biased or balanced adult sex ratios (Noireau and Dujardin 2001, Ceballos et
294 al. 2011, Abraham et al. 2011), the current data show a strong predominance of *T. guasayana* males over
295 seasons and years.

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

301 *Triatoma garciabesi* was rarely collected at the streetlight posts. This species is frequently found

302 in chicken coops, bird nests and in the rugged bark of *Prosopis* trees where chickens roosted (Canale et al.
303 2000, Turienzo and Di Iorio, 2014, Rodríguez-Planes et al. 2016). Although *T. garciabesi* outnumbered *T.*
304 *guasayana* in the peridomestic habitats of Santiago del Estero over time (Canale et al. 2000, Rodríguez-
305 Planes et al. 2016), the former was rarely collected using light traps or by householders as part of routine
306 vector surveillance; none of them colonized human sleeping quarters (Gürtler et al. 1999). Therefore, the
307 marginal light-trap catches of *T. garciabesi* in the study village and elsewhere in La Rioja (Abraham et al.
308 2011) may not necessarily imply low abundance in the surrounding habitats. The rare appearance of *T.*
309 *garciabesi* in light-trap catches and in householders' triatomine collections is in stark contrast with the
310 frequent flight behavior of *Triatoma sordida* Stål (Schofield et al. 1991) despite the former being a cryptic
311 species within the *sordida* complex (Gurgel-Gonçalves et al. 2011).

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

330 Our current results have implications for improved vector and disease control and modeling of
331 house invasion via flight dispersal: i) adult triatomines of various species attracted to public streetlights

332 are more likely to invade the neighboring houses, leading to spatial clustering of infestation in the
333 vicinities of artificial light sources, as reported for *T. dimidiata* in Yucatan (Pacheco-Tucuch et al.
334 2012) and *Rhodnius prolixus* Stål in Colombia (Jácome-Pinilla et al. 2015). Here we show the same
335 pattern is applicable to *T. infestans* in a small rural town. Because adult stages typically display the
336 largest prevalence rates of *T. cruzi* infection, such house invasions may ensue human infection risks; ii)
337 analysis of spatiotemporal infestation patterns suggested that *T. infestans* may disperse further than 1.5 km
338 in open fields (zu Dohna et al. 2009), whereas *T. dimidiata* and *R. prolixus* would be attracted by
339 streetlights up to 100 m and by house lights at least for 110 m, respectively (Barbu et al. 2010, Jácome-
340 Pinilla et al. 2015). Therefore, the exact sources of the dispersing triatomines may be quite distant to the
341 target streetlights depending on terrain features and vegetation cover; iii) The predicted increase in
342 minimum temperatures fueled by climate change is expected to widen the time window for triatomine
343 flight dispersal, and most likely increase the rates of house invasion; iv) the relationship between
344 temperatures at sunset and flight occurrence combined with local weather information may be used to
345 predict the frequency of house invasion with *T. infestans* at different locations. The time window for flight
346 dispersal was most likely restricted to the October-March period through most of the dry Argentine Chaco
347 region, but would be wider up north in the warmer Paraguayan and Bolivian Chaco, and probably
348 occurred year-round in central Brazil (Lehane et al. 1992).

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

356

357 **Acknowledgments**

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

360

361 **Financial support**

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

364

365 **References**

- 366 **Abalos, J. W., and P. Wygodzinsky. 1956.** Las vinchucas argentinas. Universidad Nacional de
367 Tucumán, Instituto de Medicina Regional, Publicación No. 705, Folleto de Divulgación No. 7. San
368 Miguel de Tucumán. 37 p.
- 369 **Abrahan, L. B., D. E. Gorla, and S. S. Catalá. 2011.** Dispersal of *Triatoma infestans* and other
370 Triatominae species in the arid Chaco of Argentina - Flying, walking or passive carriage? The
371 importance of walking females. Mem. Inst. Oswaldo Cruz 106: 232-239.
- 372 **Barbu, C., E. Dumonteil, and S. Gourbière. 2010.** Characterization of the dispersal of non-domiciliated
373 *Triatoma dimidiata* through the selection of spatially explicit models. PLoS Negl. Trop. Dis. 4:
374 e777.
- 375 **Canale, D. M., M. C. Cecere, R. Chuit, and R. E. Gürtler. 2000.** Peridomestic distribution of *Triatoma*
376 *garciabesi* and *Triatoma guasayana* in north-west Argentina. Med. Vet. Entomol. 14: 383-390.
- 377 **Carbajal de la Fuente, A. L., S. A. Minoli, C. M. Lopes, F. Noireau, C. R. Lazzari, and M. G.**
378 **Lorenzo. 2007.** Flight dispersal of the Chagas disease vectors *Triatoma brasiliensis* and *Triatoma*
379 *pseudomaculata* in northeastern Brazil. Acta Trop. 101: 115-119.
- 380 **Castro, M. C., T. V. Barrett, W. S. Santos, F. Abad-Franch, and J. A. Rafael. 2010.** Attraction of
381 Chagas disease vectors (Triatominae) to artificial light sources in the canopy of primary
382 Amazon rainforest. Mem. Inst. Oswaldo Cruz 105: 1061-1064.
- 383 **Ceballos, L. A., G. M. Vazquez-Prokopec, M. C. Cecere, P. L. Marcet, and R. E. Gürtler. 2005.**
384 Feeding rates, nutritional status and flight dispersal potential of peridomestic populations of
385 *Triatoma infestans* in rural northwestern Argentina. Acta Trop. 95: 149-159.
- 386 **Ceballos, L. A., R. V. Piccinali, P. L. Marcet, G. M. Vazquez-Prokopec, M. V. Cardinal, J.**
387 **Schachter-Broide, J. P. Dujardin, E. M. Dotson, U. Kitron, and R. E. Gürtler. 2011.**
388 Hidden sylvatic foci of the main vector of Chagas disease *Triatoma infestans*: threats to the
389 vector elimination campaign? PLoS Negl. Trop. Dis. 5: e1365.
- 390 **Cecere, M. C., M. Leporace, M. P. Fernández, J. E. Zárate, C. Moreno, R. E. Gürtler, and M. V.**
391 **Cardinal. 2016.** Host-feeding sources and infection with *Trypanosoma cruzi* of *Triatoma*
392 *infestans* and *Triatoma eratyrisiformis* (Hemiptera, Reduviidae) from the Calchaqui Valleys in
393 Northwestern Argentina. J. Med. Entomol. 53: 666-673.
- 394 **Crawford, B. A., and C. M. Kribs-Zaleta. 2013.** Vector migration and dispersal rates for sylvatic

- 395 *Trypanosoma cruzi* transmission. Ecol. Complex. 14: 145-156.
- 396 **Di Iorio, O. R. 2014.** A review of the natural history of adult Cetoniinae (Coleoptera: Scarabaeidae)
397 from Argentina and adjacent countries. Zootaxa 3790: 281-318.
- 398 **Di Iorio, O. R., and P. Turienzo. 2011.** A preliminary bibliographic survey of the insects found in
399 poultry houses from the Neotropical Region, with remarks on selected taxa shared with native
400 birds' nests. Zootaxa 2858: 1-60.
- 401 **Ekkens, D. 1981.** Nocturnal flights of *Triatoma* (Hemiptera: Reduviidae) in Sabino Canyon, Arizona.
402 I. Light collections. J. Med. Entomol. 18: 211-227.
- 403 **Galvão, C., R. U. Carcavallo, D. S. Rocha, and J. Jurberg. 2003.** A checklist of the current valid
404 species of the subfamily Triatominae Jeannel, 1919 (Hemiptera, Reduviidae) and their
405 geographical distribution, with nomenclatural and taxonomic notes. Zootaxa 202: 1-36.
- 406 **Gorla, D. E. and C. J. Schofield (1989)** Population dynamics of *Triatoma infestans* under natural
407 climatic conditions in the Argentine Chaco. Med. Vet. Entomol. 3: 179–194.
- 408 **Gurevitz, J. M., L. A. Ceballos, U. Kitron, and R. E. Gürtler. 2006.** Flight initiation of *Triatoma*
409 *infestans* (Hemiptera: Reduviidae) under natural climatic conditions. J. Med. Entomol. 43: 143-150.
- 410 **Gurevitz J. M, U. Kitron, and R. E. Gürtler. 2007.** Flight muscle dimorphism and heterogeneity in
411 flight initiation of field-collected *Triatoma infestans* (Hemiptera: Reduviidae). J. Med. Entomol. 44:
412 186-191.
- 413 **Gurgel-Gonçalves, R., J. B. C. Ferreira, A. F. Rosa, M. E. Bar, and C. Galvão. 2011.** Geometric
414 morphometrics and ecological niche modelling for delimitation of near-sibling triatomine species.
415 Med Vet Entomol 25: 84–93.
- 416 **Gürtler, R. E., M. C. Cecere, D. Canale, M. B. Castañera, R. Chuit, and J. E. Cohen. 1999.**
417 Monitoring house reinfestation by vectors of Chagas disease: a comparative trial of detection
418 methods during a four-year follow-up. Acta Trop. 72: 213-234.
- 419 **Gürtler, R. E., M. C. Cecere, M. P. Fernández, G. M. Vazquez-Prokopec, L. A. Ceballos, J. M.**
420 **Gurevitz, U. Kitron, and J. E. Cohen. 2014.** Key source habitats and potential dispersal of
421 *Triatoma infestans* populations in northwestern Argentina: Implications for vector control.
422 PLoS Negl. Trop. Dis. 8: e3238.

- 423 **Jácome-Pinilla, D., E. Hincapie-Peñaloza, M. I. Ortiz, J. D. Ramírez, F. Guhl, and J. Molina.**
424 **2015.** Risks associated with dispersive nocturnal flights of sylvatic Triatominae to artificial
425 lights in a model house in the northeastern plains of Colombia. *Parasit. Vectors* 19: 600.
- 426 **Lazzari, C. R. 1992.** Circadian organization of locomotion activity in the haematophagous bug
427 *Triatoma infestans*. *J. Insect Physiol.* 38: 895-903.
- 428 **Lehane, M. J., P. K. McEwen, C. J. Whitaker, and C. J. Schofield. 1992.** The role of temperature and
429 nutritional status in flight initiation by *Triatoma infestans*. *Acta Trop.* 52: 27-38.
- 430 **Lent, H., and P. Wygodzinsky. 1979.** Revision of the Triatominae (Hemiptera, Reduviidae) and their
431 significance as vectors of Chagas' disease. *Bull. Am. Mus. Nat. Hist.* 163: 123-520.
- 432 **Levy, M. Z., V. R. Quispe-Machaca, J. L. Ylla-Velasquez, J. M. Richards, B. Rath, K. Borrini-**
433 **Mayori, L. A. Waller, J. G. Cornejo del Carpio, E. Cordova Benzaquen, F. E. McKenzie, R.**
434 **A. Wirtz, J. H. Maguire, R. H. Gilman, and C. Bern. 2008.** Impregnated netting slows
435 infestation by *Triatoma infestans*. *Am. J. Trop. Med. Hyg.* 79: 528-534.
- 436 **Minoli, S. A., and C. R. Lazzari. 2006.** Take-off activity and orientation of triatomines (Heteroptera:
437 Reduviidae) in relation to the presence of artificial lights. *Acta Trop.* 97: 324-330.
- 438 **Noireau, F., and J. P. Dujardin. 2001.** Flight and nutritional status of sylvatic *Triatoma sordida* and
439 *Triatoma guasayana*. *Mem. Inst. Oswaldo Cruz* 96: 385-389.
- 440 **Pacheco-Tucuch, F. S., M. J. Ramirez-Sierra, S. Gourbière, and E. Dumonteil. 2012.** Public street
441 lights increase house infestation by the Chagas disease vector *Triatoma dimidiata*. *PLoS One* 7:
442 e36207.
- 443 **Rodríguez-Planes, L. I., G. M. Vazquez-Prokopec, M. C. Cecere, D. M. Canale, and R. E.**
444 **Gürtler. 2016.** Selective insecticide applications directed against *Triatoma infestans*
445 (Hemiptera: Reduviidae) affected a nontarget secondary vector of Chagas disease, *Triatoma*
446 *garciabesi*. *J. Med. Entomol.* 53: 144-151.
- 447 **Sainz, A. C., L. V. Mauro, E. N. Moriyama, and B. A. García. 2004.** Phylogeny of triatomine
448 vectors of *Trypanosoma cruzi* suggested by mitochondrial DNA sequences. *Genetica* 121: 229-
449 240.
- 450 **Schofield, C. J., M. J. Lehane, P. McEwen, S. S. Catalá, and D. E. Gorla. 1991.** Dispersive flight by
451 *Triatoma sordida*. *Trans. R. Soc. Trop. Med. Hyg.* 85: 676-678.
- 452 **Schofield, C. J., M. J. Lehane, P. McEwen, S. S. Catalá, and D. E. Gorla. 1992.** Dispersive flight by

- 453 *Triatoma infestans* under natural climatic conditions in Argentina. Med. Vet. Entomol. 6: 51-
454 56.
- 455 **Sjögren, R. D., and R. E. Ryckman. 1966.** Epizootiology of *Trypanosoma cruzi* in southwestern
456 North America Part VIII: nocturnal flights of *Triatoma protracta* (Uhler) as indicated by
457 collections at black light traps. J. Med. Entomol. 3: 81-92.
- 458 **Stata Corp. 2017.** Stata Statistical Software. Release 14.2. College Station: Stata Corporation.
- 459 **Stroppa, M. M., M. S. Lagunas, C. S. Carriazo, B. A. Garcia, G. Iraola, Y. Panzera, and N. M.
460 Gerez de Burgos. 2013.** Differential expression of glycerol-3-phosphate dehydrogenase isoforms
461 in flight muscles of the Chagas disease vector *Triatoma infestans* (Hemiptera, Reduviidae). Am. J.
462 Trop. Med. Hyg. 88: 1146-1151.
- 463 **Turienzo, P., and O. R. Di Iorio. 2014.** Insects in birds' nests from Argentina. *Pseudoseisura lophotes*
464 Reichenbach, 1853 and *Anumbius annumbi* (Vieillot, 1817) (Aves: Furnariidae), hosts of
465 *Triatoma platensis* Neiva, 1913 (Hemiptera: Reduviidae: Triatominae). Zootaxa 3766: 1-82.
- 466 **Vazquez-Prokopec, G. M., L. A. Ceballos, P. L. Marcet, M. C. Cecere, M. V. Cardinal, U. Kitron,
467 and R. E. Gürtler. 2006.** Seasonal variations in active dispersal of natural populations of
468 *Triatoma infestans* in rural north-western Argentina. Med. Vet. Entomol. 20: 273-279.
- 469 **Vazquez-Prokopec, G. M., M. C. Cecere, U. Kitron, and R. E. Gürtler. 2008.** Environmental and
470 demographic factors determining the spatial distribution of *Triatoma guasayana* in peridomestic
471 and semi-sylvatic habitats of rural northwestern Argentina. Med. Vet. Entomol. 22: 273-282.
- 472 **Vezzani, D., N. J. Schweigmann, S. M. Pietrokowsky, and C. Wisnivesky-Colli. 2001.**
473 Characterization of *Triatoma guasayana* biotopes in a hardwood forest of Santiago del Estero,
474 Argentina. Mem. Inst. Oswaldo Cruz 96: 459-466.
- 475 **Wygodzinsky, P., and J. W. Abalos. 1950.** *Triatoma guasayana* Wygodzinsky y Abalos, 1949, con
476 notas sobre especies vecinas (Triatominae, Reduviidae, Hemiptera). An. Inst. Med. Reg. Tucumán
477 3: 53-73.
- 478 **Yeo, M., N. Acosta, M. Llewellyn, H. Sánchez, S. Adamson, G. A. Miles, E. López, N. González, J.
479 S. Patterson, M. W. Gaunt, A. R. de Arias, and M. A. Miles. 2005.** Origins of Chagas
480 disease: *Didelphis* species are natural hosts of *Trypanosoma cruzi* I and armadillos hosts of
481 *Trypanosoma cruzi* II, including hybrids. Int J Parasitol. 35: 225-233.

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

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

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

490

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

494

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

498

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

Table 1. Overall catch of adult *Triatoma* (Hemiptera: Reduviidae) 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				Mean (SD) catch per 100 light trap- nights
		Males	Females	Total	% females	
<i>T. guasayana</i>	75 (17.6)	94	28	122	23.0	28.7 (78.8)
<i>T. infestans</i>	60 (14.1)	47	42	89	47.2	20.9 (63.4)
<i>T. eratyrisiformis</i>	51 (12.0)	49	23	72	31.9	16.9 (53.2)
<i>T. garciabesi</i>	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. eratyrisiformis* 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	
<i>Triatoma infestans</i>	Yes	11	24	$P = 1.00$
	No	25	365	
<i>Triatoma guasayana</i>	Yes	9	51	$P < 0.0001$
	No	15	350	
<i>Triatoma eratyrisiformis</i>	Yes	10	29	$P = 0.012$
	No	12	374	

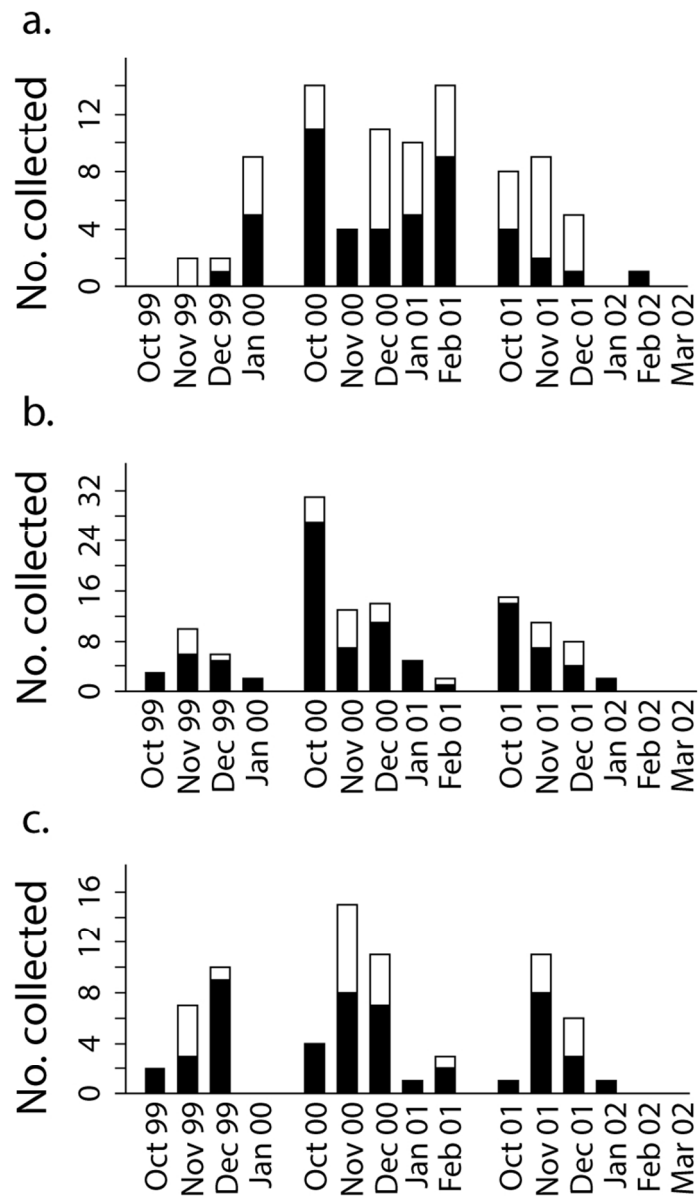


Figure 1. Catch of *Triatoma infestans* (a), *Triatoma guasayana* (b) and *Triatoma eratyrusiformis* (c) adult triatomines at the streetlight posts by sex, month and year at Los Molinos village, 1999-2002 period. Black bars are for males, white bars for females.

73x116mm (300 x 300 DPI)

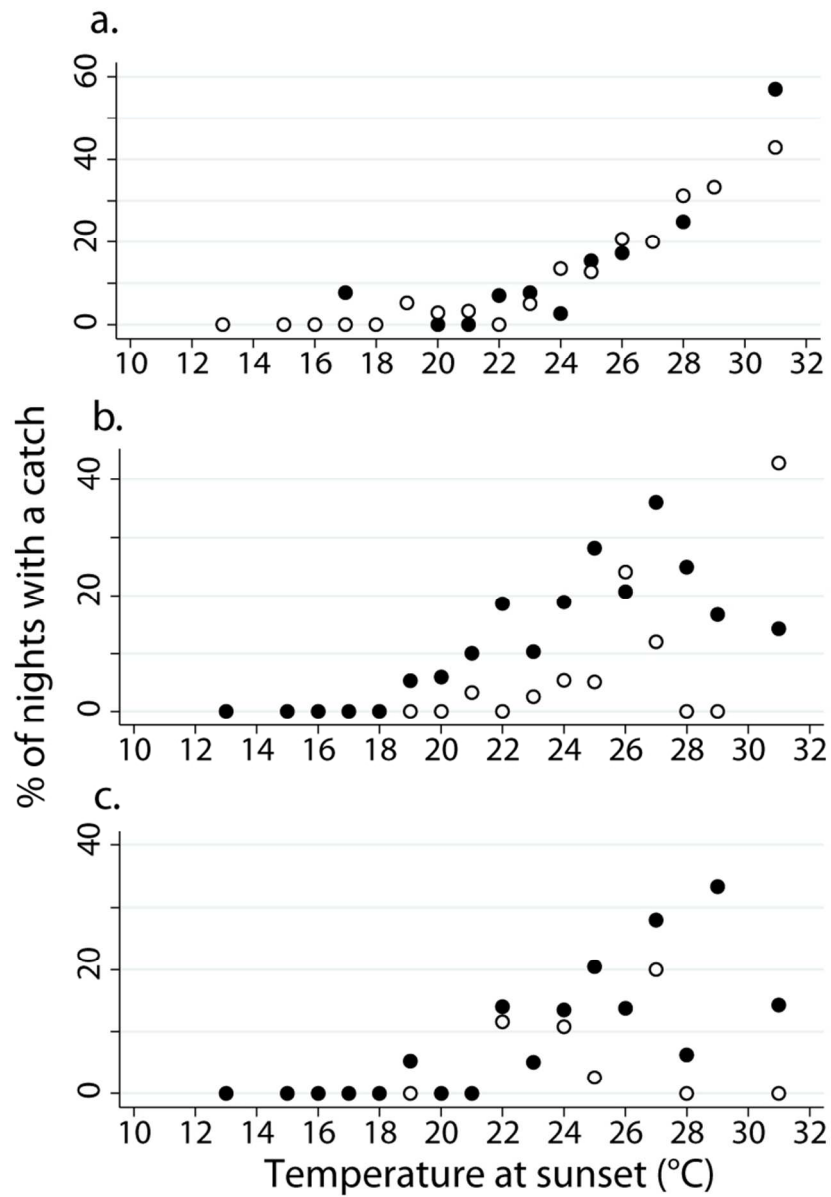


Figure 2. Percentage of nights in which at least an adult *Triatoma infestans* (a), *Triatoma guasayana* (b) or *Triatoma eratyrusiformis* (c) was caught at the streetlight posts according to sex and temperature at sunset in Los Molinos village, 1999-2002 period. Solid circles are males, white circles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

68x99mm (300 x 300 DPI)

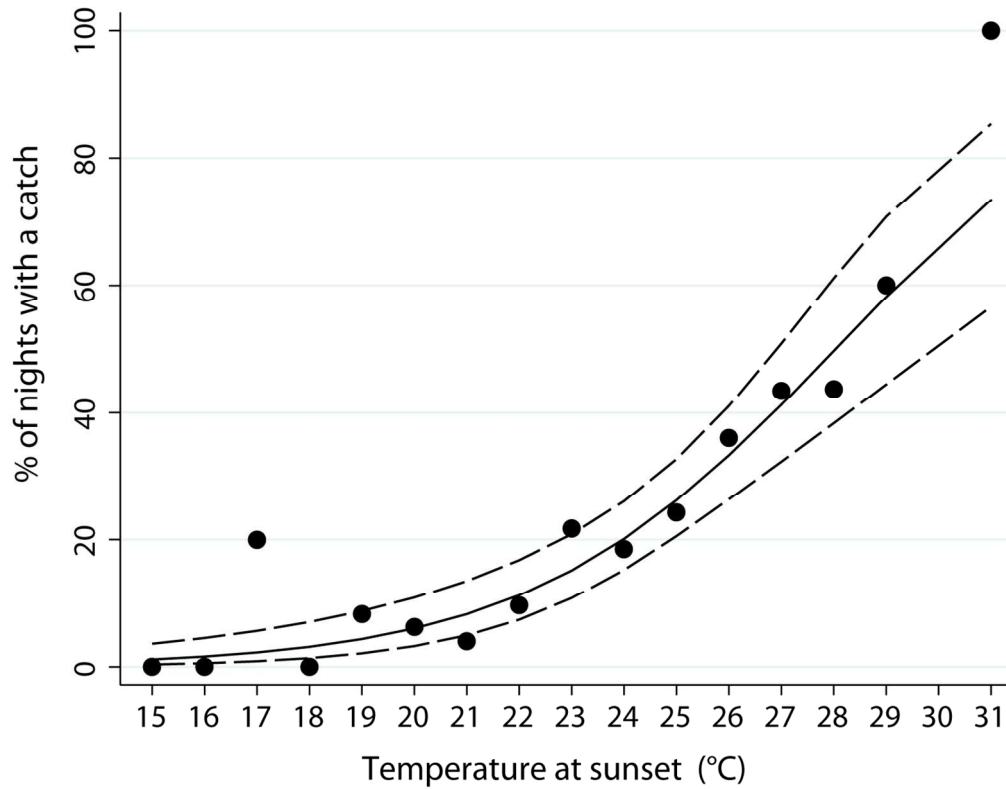


Figure 3. Observed (black dots) and predicted (solid line) percentage of nights in which at least one adult *Triatoma infestans* was caught at the streetlight posts in non-rainy days according to temperature at sunset in Los Molinos village, 1999-2002 period. Dashed lines are the upper and lower bounds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

124x97mm (300 x 300 DPI)

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

4

5 Sampling, Distribution, Dispersal

6

7

8

9

10

11

12

13

14

15

16

17 R. E. Gürtler

18 Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas.

19 Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBEA), Facultad de

20 Ciencias Exactas y Naturales, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina.

21 Tel/fax: (5411) 4576-3318

22 E-mail: gurtler@ege.fcen.uba.ar

23

24

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

27

28 Osvaldo Di Iorio,^{1,†} and Ricardo E. Gürtler^{2,3}

29

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

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

35 ³ Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad de Buenos Aires. Instituto de
36 Ecología, Genética y Evolución de Buenos Aires (IEGEB), Ciudad Universitaria, C1428EHA,
37 Buenos Aires, Argentina.

38

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 eratyrisiformis* 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. **BugTriatomine** 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 eratyrisiformis*, *Trypanosoma cruzi*, vector control, temperature, weather

62 Active dispersal of Triatominae (Hemiptera: Reduviidae) determines the spatial patterns of house
63 invasion and vector infection with *Trypanosoma cruzi* Chagas (Kinetoplastida: Trypanosomatidae), the
64 etiologic agent of Chagas disease (Vazquez-Prokopec et al. 2006, Levy et al. 2008, Barbu et al. 2011).
65 These patterns, derived from environmental conditions combined with nutritional and reproductive status,
66 modify the flight activity of triatomine s-bugs (Sjögren and Ryckman 1966, Ekkens 1981, Lehane et al.
67 1992, Schofield et al. 1992, Gurevitz et al. 2006). For example, higher triatomine densities per host
68 would impair the insects' nutritional status (measured by body weight-to-length ratios, W:L), increase
69 the likelihood of flight initiation, and contribute to the regulation of local population density (Lehane et
70 al. 1992, Schofield et al. 1992). Artificial light sources usually attract adult triatomines of many species
71 and favor house invasion (Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2006, Minoli and Lazzari
72 2006, Carbajal de la Fuente et al. 2007, Castro et al. 2010, Abrahan et al. 2011, Pacheco-Tucuch et al.
73 2012, Jácome-Pinilla et al. 2015).

74 Seasonal variations in flight dispersal are fairly common in Triatominae, with flights occurring
75 within the first 2-3 h after sunset in most of the species investigated in the field (Sjögren and Ryckman
76 1966, Schofield et al. 1992, Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2006). For example,
77 the flight activity of *Triatoma protracta* Uhler and *Triatoma rubida* Neiva peaked in summer in the
78 Arizona desert (Sjögren and Ryckman 1966, Ekkens 1981), whereas flights of *Triatoma dimidiata*
79 Latreille and other species peaked in late spring (dry season) in the forests of Costa Rica (Zeledón et al.
80 2001). Catches of nine species of Triatominae in a light trap located at canopy level peaked in October-
81 December in the Amazon rainforest (Castro et al. 2010). *Triatoma guasayana* Wygodzinsky and
82 Abalos, a sylvatic or peridomestic species in the dry Chaco ecoregion extending over sections of
83 Argentina, Bolivia and Paraguay, apparently had a distinct flight dispersal window in spring (Gürtler et
84 al. 1999, Noireau and Dujardin 2001, Vazquez-Prokopec et al. 2008, Abrahan et al. 2011, Ceballos et
85 al. 2011), but light-trap efforts at other times were rather sparse.

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

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

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

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

111 112 **Materials and methods**

113 **Study area.** Field work was conducted at Los Molinos (28° 43' 60'' S, 66° 55' 60'' W), a small rural
114 village with approximately 600 people located at 1,254 m above sea level in an arid zone of La Rioja
115 province (Departamento Castro Barros), western Argentina. The village was situated on low terrain,
116 which determined the virtual absence of strong winds. Most of Los Molinos village comprised abandoned
117 orchards with artificial irrigation and houses with well-plastered brick walls and tin, cement or tiled roofs.
118 Householders usually raised chickens, goats, dogs and pigs. Estates were separated by old fences made
119 with piled rocks. High-pressure mercury-vapor lamps (250 watts, which radiate predominantly in the
120 ultraviolet and visible regions of the optical range) were used for street lighting. Mercury vapor lamps are
121 energetically more efficient than incandescent and most fluorescent lights, with luminous efficacies of 35

122 to 65 lumens/watt (i.e., a high-intensity, clear white light output). Local residents reported that Chagas
123 vector control programs had not sprayed their houses with insecticide between 1991 and 2002. The
124 local climate was markedly seasonal, with dry (fall-winter) and wet (spring-summer) periods.

125 Mean monthly temperatures at sunset increased from 19.9°C in October to 23.1-23.6°C between
126 December and February, with substantial variations within the same month over the study period (Di Iorio
127 2014). Monthly temperatures during the second summer (December 2000-February 2001) were on
128 average 2-3°C higher than in the previous or subsequent years. Monthly minima at sunset ranged from
129 11.0 to 13.3°C between October and December, and increased to 16.7-17.3°C between January and
130 March throughout the study period. The percentage of days with rainfall was much less frequent in
131 October (mean, 7.5%; range, 0.0-12.9%) or November (10.8%; 9.7-12.9%) than in December (25.8%;
132 12.9-41.9%), January (36.6%; 32.3-41.8%) and February (25.8%; 19.4-32.3%). The second spring-
133 summer period (2000-2001) was unusually wet relative to the previous and subsequent years, with **much**
134 **greater+100%** herbaceous coverage and **peak**-insect abundance in several areas.

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

147 Temperatures were recorded exactly at sunset with a digital thermo-hygrometer, except in October
148 1999 and February 2000, and with a red-colored alcohol thermometer in November and December 1999
149 for a total of 425 days. These data were compared with those collected by a weather station at La Rioja
150 airport at 500 m of altitude, approximately 200 km from Los Molinos village (Servicio Meteorológico
151 Nacional, unpublished data). Both data sets showed an average difference of 6.8°C at sunset (N = 392

152 observations) and therefore the airport weather station data were excluded from further consideration.

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

170

171

Results

172 A total of 288 specimens of four species of Triatominae were captured at the streetlight posts between
173 1999 and 2002 (Table 1). Adult triatomines were spotted resting on the substrate or walking within the
174 light cone when they were captured. *Triatoma guasayana* was the most abundant species, representing
175 42.4% of the total catch, followed by *T. infestans* (30.9%) and *T. eratyrsiformis* (25.0%). Only 5 (1.7%)
176 specimens of *T. garciabesi* were caught. This ranking also held for the average catch per trap-night and
177 the frequency of sample nights in which each species was collected: *T. guasayana* was caught in 75
178 (17.6%) of 425 sample nights, closely followed by *T. infestans* (14.1%), *T. eratyrsiformis* (12.0%), and
179 *T. garciabesi* (1.2%). Overall adult sex ratios were fairly balanced in *T. infestans* (47.2% of females) and
180 highly skewed toward males in the other species. All specimens were unfed when they were captured as
181 confirmed by visual inspection.

182 Most of the catches of *T. infestans* comprised only one insect (on 44 occasions); 2, 3, 4 and ≥ 5
183 insects were collected during the same night on 9, 3, 2 and 2 occasions, respectively. The frequency
184 distributions of 1, 2, 3, 4 and ≥ 5 adult triatomines collected were 50, 15, 4, 1 and 5 for *T. guasayana*, and
185 37, 9, 3, 2 and 0 for *T. eratyrisiformis*, respectively. Male *T. guasayana* and *T. eratyrisiformis* were
186 caught significantly more often than females of the corresponding species during the same night, but this
187 relationship did not hold for *T. infestans* (Table 2).

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

195 Nearly all catches of flight-dispersing triatomines (282 of 288, 97.9%) occurred when
196 temperatures at sunset were above 20°C (range, 16.6-31.7°C) (Fig. 2A-C). Only in one (3%) of the 92
197 rainy days recorded throughout the study was an adult triatomine caught at the streetlights. For *T.*
198 *infestans*, the minimum temperature at sunset at which an adult triatomine was captured was 16.6°C (in
199 October) and the maximum was at 31.7°C (in February) (Fig. 2A). The logit of the proportion of sample
200 nights in which at least an adult *T. infestans* was caught on a non-rainy day (y) increased significantly with
201 temperature at sunset (x_1) and was modified by collection month of insect (x_{2-5}) ($y = 0.4312$ (SE, 0.0685)
202 $x_1 - 0.2617$ (SE, 0.5319) $x_2 - 0.5706$ (SE, 0.4977) $x_3 + 0.2515$ (SE, 0.4947) $x_4 + 1.4126$ (SE, 0.5593) $x_5 -$
203 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,
204 February, November and October, respectively, taking December as the reference level). The temperature-
205 adjusted relative odds of a catch was fourfold greater in October than in December (OR = 4.11, 95%
206 confidence interval, 1.37-12.29). The model displayed a close fit to the data (Hosmer-Lemeshow $\chi^2 =$
207 5.66 , 8 df , $P = 0.685$), with total observations for each quantile ranging from 26 to 33. The area under the
208 ROC curve was 0.815. Model coefficients were not significantly modified by sex of insect. Figure- 3
209 shows the observed and predicted percentage of nights with a catch (both sexes combined) as a function of
210 temperatures at sunset between 15 and 31°C; only two data points at both extremes deviated significantly
211 from predictions.

212 For *T. guasayana*, nearly 50% of all specimens were caught during the second season (Fig. 1B).
213 The flight period spanned from October to December, with very few specimens collected from January to
214 February and peak catches in October. The overall sex ratio was consistently male-biased. The minimum
215 and maximum temperatures at which specimens were captured were 19.4 and 30.7°C, respectively (Fig.
216 2B). The logistic regression model displayed a close fit to the observed proportion of nights in which at
217 least a *T. guasayana* was caught (Hosmer-Lemeshow $\chi^2 = 4.91$, 8 df, $P = 0.767$), and the area under the
218 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) $x_3 +$
219 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, $P < 0.0001$,
220 pseudo $R^2 = 0.224$).

221 For *T. eratyrisiformis*, the flight period also extended from October to December, with peak
222 catches in November-December and very few specimens collected over January and February (Fig. 1C).
223 The overall sex ratio was male-biased throughout the observation period. The minimum and maximum
224 temperatures at which specimens were captured were 19.4 and 29.8°C, respectively (Fig. 2C). As with the
225 other species, the logistic model displayed a close fit to the data (Hosmer-Lemeshow $\chi^2 = 10.46$, 8 df, $P =$
226 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)
227 $x_2 - 3.2070$ (SE, 1.0640) $x_3 + 0.8151$ (SE, 0.4513) $x_4 - 0.2610$ (SE, 0.6110) $x_5 - 9.5031$ (SE, 1.7074); χ^2
228 $= 60.49$, df = 5, $P < 0.0001$, pseudo $R^2 = 0.242$).

229

230

Discussion

231 The most important results of our study are related to the seasonal timing of flight dispersal in *T. infestans*
232 and other triatomines, and the close relationship between flight occurrence toward streetlights and
233 temperatures at sunset. Our study documents a large number of dispersing adults of *T. infestans*, which
234 were mainly collected during spring of the second year (the warmest over the 3-yr period), with greater
235 catches in October. This pattern is consistent with the outcomes of a detailed time-series analysis of site
236 occupancy of *T. infestans* over 10 years (zu Dohna et al. 2009), and habitat-specific flight indices based on
237 blood-feeding patterns and other population attributes in the dry Chaco mid-spring (Gürtler et al. 2014).
238 These patterns were not supported by other light-trap surveys with sparse sampling during spring
239 (Vazquez-Prokopec et al. 2006, Abraham et al. 2011). Taken together, the evidence strongly refutes that
240 flight dispersal of *T. infestans* is rare in spring. We note, however, that weather conditions (including
241 temperatures at sunset) were different in Los Molinos, situated in arid western Argentina at 1,200 m of

242 altitude, than in the dry Chaco region of Santiago del Estero at less than 200 m of elevation. Insect flights
243 at dusk declined steeply during March at the higher altitude of Los Molinos (Di Iorio, unpublished data),
244 unlike in the dry Chaco studies. The spring flight dispersal window is likely to be caused by a chain of
245 events associated with rising spring temperatures and increasing day length after the cold winter months
246 in which triatomines remained metabolically inactive, followed by peak adult emergence (Gorla and
247 Schofield 1980) and the 3- or 4-wk period needed for flight muscle development (Gurevitz et al. 2006).
248 Spring flights are finally made evident under suitable weather conditions.

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

255 | Nearly all specimens of the four triatomine species flew at temperatures at sunset above 20°C,
256 suggesting a putative threshold for flight dispersal around 17-18°C. This estimate is consistent with
257 experimental evidence showing that the spontaneous locomotory activity of male *T. infestans* would be
258 strongly reduced below 20°C (Lazzari 1992), whereas flight initiation steeply increased from 0% at 15°C,
259 6.2% at 18°C to 35.2% at 22°C (after pooling data for both sexes and repeat experiments at given
260 temperatures in Table 1 of Lehane et al. 1992). Such thresholds may be related to the activity of the two
261 isoforms of the enzyme glycerol-3-phosphate dehydrogenase, which is crucially linked to flight
262 metabolism in triatomines (Stroppa et al. 2013).

263 The relative odds of flight occurrence of *T. infestans* toward the streetlights increased highly
264 significantly with increasing temperature at sunset in both sexes. Similarly, flight initiation probabilities
265 increased sigmoidally with increasing temperature at sunset in caged experimental huts under natural
266 climatic conditions, although females displayed consistently higher flight probabilities than males after
267 adjusting for W:L effects (Gurevitz et al. 2006). Both metrics are intimately linked by the same
268 temperature-dependent, binomial process: flight initiation probabilities most likely generated the
269 proportions of nights in which at least one *T. infestans* flew toward the streetlights. In the experimental
270 huts, the asymptote recorded over 25-30°C was in part determined by the large fraction of adult
271 | ~~bug~~triatomines lacking flight muscles (Gurevitz et al. 2007) whereas in the current study there was no

272 asymptote. Here the proportion of females among flight-dispersing adults of *T. infestans* (47.2%) was
273 substantially larger than in light-trap surveys conducted in Santiago del Estero (33.3% of 21 adults)
274 (Vazquez-Prokopec et al. 2006) and in La Rioja (37.5% of 8 adults) (Abrahan et al. 2011). For
275 comparison, the mean percentage of adult females over domestic and peridomestic populations of *T.*
276 *infestans* averaged 41.7% and varied substantially among ecotopes in the mid-spring of Santiago del
277 Estero (Gürtler et al. 2014).

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

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

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

302 *Triatoma garciabesi* was rarely collected at the streetlight posts. This species is frequently found
303 in chicken coops, bird nests and in the rugged bark of *Prosopis* trees where chickens roosted (Canale et al.
304 2000, Turienzo and Di Iorio, 2014, Rodríguez-Planes et al. 2016). Although *T. garciabesi* outnumbered *T.*
305 *guasayana* in the peridomestic habitats of Santiago del Estero over time (Canale et al. 2000, Rodríguez-
306 Planes et al. 2016), the former was rarely collected using light traps or by householders as part of routine
307 vector surveillance; none of them colonized human sleeping quarters (Gürtler et al. 1999). Therefore, the
308 marginal light-trap catches of *T. garciabesi* in the study village and elsewhere in La Rioja (Abraham et al.
309 2011) may not necessarily imply low abundance in the surrounding habitats. The rare appearance of *T.*
310 *garciabesi* in light-trap catches and in householders' bugtriatomine collections is in stark contrast with the
311 frequent flight behavior of *Triatoma sordida* Stål (Schofield et al. 1991) despite the former being a cryptic
312 species within the *sordida* complex (Gurgel-Gonçalves et al. 2011).

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

331 Our current results have implications for improved vector and disease control and modeling of
332 house invasion via flight dispersal: i) adult triatomines of various species attracted to public streetlights
333 are more likely to invade the neighboring houses, leading to spatial clustering of infestation in the
334 vicinities of artificial light sources, as reported for *T. dimidiata* in Yucatan (Pacheco-Tucuch et al.
335 2012) and *Rhodnius prolixus* Stål in Colombia (Jácome-Pinilla et al. 2015). Here we show the same
336 pattern is applicable to *T. infestans* in a small rural town. Because adult stages typically display the
337 largest prevalence rates of *T. cruzi* infection, such house invasions may ensue human infection risks; ii)
338 analysis of spatiotemporal infestation patterns suggested that *T. infestans* may disperse further than 1.5 km
339 in open fields (zu Dohna et al. 2009), whereas *T. dimidiata* and *R. prolixus* would be attracted by
340 streetlights up to 100 m and by house lights at least for 110 m, respectively (Barbu et al. 2010, Jácome-
341 Pinilla et al. 2015). Therefore, the exact sources of the dispersing **bugtriatomines** may be quite distant to
342 the target streetlights depending on terrain features and vegetation cover; iii) The predicted increase in
343 minimum temperatures fueled by climate change is expected to widen the time window for triatomine
344 flight dispersal, and most likely increase the rates of house invasion; iv) the relationship between
345 temperatures at sunset and flight occurrence combined with local weather information may be used to
346 predict the frequency of house invasion with *T. infestans* at different locations. The time window for flight
347 dispersal was most likely restricted to the October-March period through most of the dry Argentine Chaco
348 region, but would be wider up north in the warmer Paraguayan and Bolivian Chaco, and probably
349 occurred year-round in central Brazil (Lehane et al. 1992).

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

357

358 Acknowledgments

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

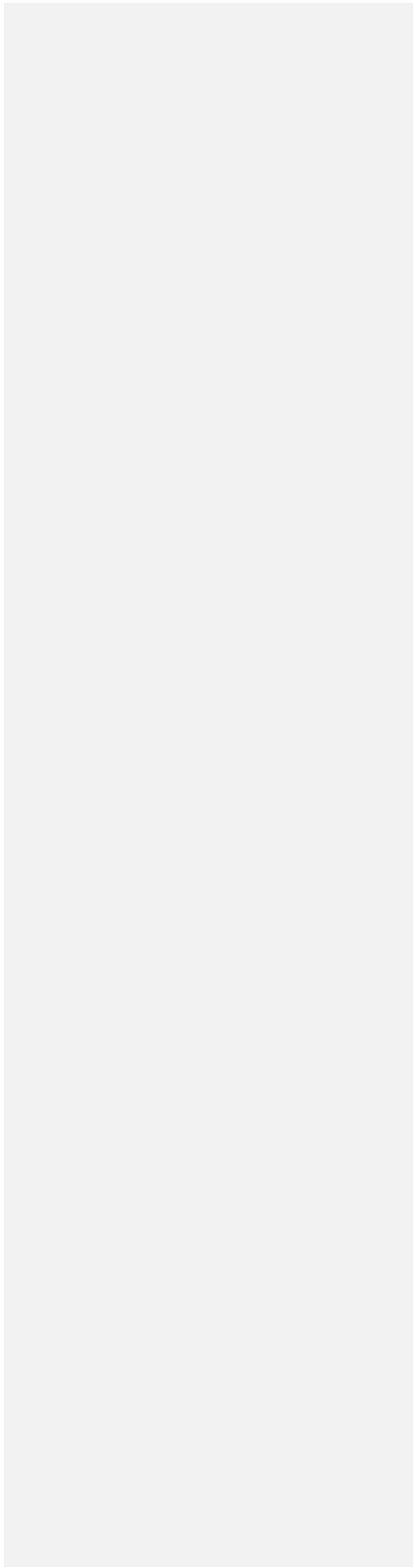
360 Rioja Airport, Lucía Rodríguez-Planes and Paula M. Gürtler for helpful assistance and comments.

361

362 **Financial support**

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

365



References

- 366
367 **Abalos, J. W., and P. Wygodzinsky. 1956.** Las vinchucas argentinas. Universidad Nacional de
368 Tucumán, Instituto de Medicina Regional, Publicación No. 705, Folleto de Divulgación No. 7. San
369 Miguel de Tucumán. 37 p.
- 370 **Abrahan, L. B., D. E. Gorla, and S. S. Catalá. 2011.** Dispersal of *Triatoma infestans* and other
371 Triatominae species in the arid Chaco of Argentina - Flying, walking or passive carriage? The
372 importance of walking females. Mem. Inst. Oswaldo Cruz 106: 232-239.
- 373 **Barbu, C., E. Dumonteil, and S. Gourbière. 2010.** Characterization of the dispersal of non-domiciliated
374 *Triatoma dimidiata* through the selection of spatially explicit models. PLoS Negl. Trop. Dis. 4:
375 e777.
- 376 **Canale, D. M., M. C. Cecere, R. Chuit, and R. E. Gürtler. 2000.** Peridomestic distribution of *Triatoma*
377 *garciabesi* and *Triatoma guasayana* in north-west Argentina. Med. Vet. Entomol. 14: 383-390.
- 378 **Carbajal de la Fuente, A. L., S. A. Minoli, C. M. Lopes, F. Noireau, C. R. Lazzari, and M. G.**
379 **Lorenzo. 2007.** Flight dispersal of the Chagas disease vectors *Triatoma brasiliensis* and *Triatoma*
380 *pseudomaculata* in northeastern Brazil. Acta Trop. 101: 115-119.
- 381 **Castro, M. C., T. V. Barrett, W. S. Santos, F. Abad-Franch, and J. A. Rafael. 2010.** Attraction of
382 Chagas disease vectors (Triatominae) to artificial light sources in the canopy of primary
383 Amazon rainforest. Mem. Inst. Oswaldo Cruz 105: 1061-1064.
- 384 **Ceballos, L. A., G. M. Vazquez-Prokopec, M. C. Cecere, P. L. Marcet, and R. E. Gürtler. 2005.**
385 Feeding rates, nutritional status and flight dispersal potential of peridomestic populations of
386 *Triatoma infestans* in rural northwestern Argentina. Acta Trop. 95: 149-159.
- 387 **Ceballos, L. A., R. V. Piccinali, P. L. Marcet, G. M. Vazquez-Prokopec, M. V. Cardinal, J.**
388 **Schachter-Broide, J. P. Dujardin, E. M. Dotson, U. Kitron, and R. E. Gürtler. 2011.**
389 Hidden sylvatic foci of the main vector of Chagas disease *Triatoma infestans*: threats to the
390 vector elimination campaign? PLoS Negl. Trop. Dis. 5: e1365.
- 391 **Cecere, M. C., M. Leporace, M. P. Fernández, J. E. Zárate, C. Moreno, R. E. Gürtler, and M. V.**
392 **Cardinal. 2016.** Host-feeding sources and infection with *Trypanosoma cruzi* of *Triatoma*
393 *infestans* and *Triatoma eratyrisiformis* (Hemiptera, Reduviidae) from the Calchaqui Valleys in
394 Northwestern Argentina. J. Med. Entomol. 53: 666-673.
- 395 **Crawford, B. A., and C. M. Kribs-Zaleta. 2013.** Vector migration and dispersal rates for sylvatic

- 396 *Trypanosoma cruzi* transmission. Ecol. Complex. 14: 145-156.
- 397 **Di Iorio, O. R. 2014.** A review of the natural history of adult Cetoniinae (Coleoptera: Scarabaeidae)
398 from Argentina and adjacent countries. Zootaxa 3790: 281-318.
- 399 **Di Iorio, O. R., and P. Turienzo. 2011.** A preliminary bibliographic survey of the insects found in
400 poultry houses from the Neotropical Region, with remarks on selected taxa shared with native
401 birds' nests. Zootaxa 2858: 1-60.
- 402 **Ekkens, D. 1981.** Nocturnal flights of *Triatoma* (Hemiptera: Reduviidae) in Sabino Canyon, Arizona.
403 I. Light collections. J. Med. Entomol. 18: 211-227.
- 404 **Galvão, C., R. U. Carcavallo, D. S. Rocha, and J. Jurberg. 2003.** A checklist of the current valid
405 species of the subfamily Triatominae Jeannel, 1919 (Hemiptera, Reduviidae) and their
406 geographical distribution, with nomenclatural and taxonomic notes. Zootaxa 202: 1-36.
- 407 **Gorla, D. E. and C. J. Schofield (1989)** Population dynamics of *Triatoma infestans* under natural
408 climatic conditions in the Argentine Chaco. Med. Vet. Entomol. 3: 179-194.
- 409 **Gurevitz, J. M., L. A. Ceballos, U. Kitron, and R. E. Gürtler. 2006.** Flight initiation of *Triatoma*
410 *infestans* (Hemiptera: Reduviidae) under natural climatic conditions. J. Med. Entomol. 43: 143-150.
- 411 **Gurevitz J. M, U. Kitron, and R. E. Gürtler. 2007.** Flight muscle dimorphism and heterogeneity in
412 flight initiation of field-collected *Triatoma infestans* (Hemiptera: Reduviidae). J. Med. Entomol. 44:
413 186-191.
- 414 **Gurgel-Gonçalves, R., J. B. C. Ferreira, A. F. Rosa, M. E. Bar, and C. Galvão. 2011.** Geometric
415 morphometrics and ecological niche modelling for delimitation of near-sibling triatomine species.
416 Med Vet Entomol 25: 84-93.
- 417 **Gürtler, R. E., M. C. Cecere, D. Canale, M. B. Castañera, R. Chuit, and J. E. Cohen. 1999.**
418 Monitoring house reinfestation by vectors of Chagas disease: a comparative trial of detection
419 methods during a four-year follow-up. Acta Trop. 72: 213-234.
- 420 **Gürtler, R. E., M. C. Cecere, M. P. Fernández, G. M. Vazquez-Prokopec, L. A. Ceballos, J. M.**
421 **Gurevitz, U. Kitron, and J. E. Cohen. 2014.** Key source habitats and potential dispersal of
422 *Triatoma infestans* populations in northwestern Argentina: Implications for vector control.
423 PLoS Negl. Trop. Dis. 8: e3238.

- 424 **Jácome-Pinilla, D., E. Hincapie-Peñaloza, M. I. Ortiz, J. D. Ramírez, F. Guhl, and J. Molina.**
425 **2015.** Risks associated with dispersive nocturnal flights of sylvatic Triatominae to artificial
426 lights in a model house in the northeastern plains of Colombia. *Parasit. Vectors* 19: 600.
- 427 **Lazzari, C. R. 1992.** Circadian organization of locomotion activity in the haematophagous bug
428 *Triatoma infestans*. *J. Insect Physiol.* 38: 895-903.
- 429 **Lehane, M. J., P. K. McEwen, C. J. Whitaker, and C. J. Schofield. 1992.** The role of temperature and
430 nutritional status in flight initiation by *Triatoma infestans*. *Acta Trop.* 52: 27-38.
- 431 **Lent, H., and P. Wygodzinsky. 1979.** Revision of the Triatominae (Hemiptera, Reduviidae) and their
432 significance as vectors of Chagas' disease. *Bull. Am. Mus. Nat. Hist.* 163: 123-520.
- 433 **Levy, M. Z., V. R. Quíspe-Machaca, J. L. Ylla-Velasquez, J. M. Richards, B. Rath, K. Borrini-**
434 **Mayori, L. A. Waller, J. G. Cornejo del Carpio, E. Cordova Benzaquen, F. E. McKenzie, R.**
435 **A. Wirtz, J. H. Maguire, R. H. Gilman, and C. Bern. 2008.** Impregnated netting slows
436 infestation by *Triatoma infestans*. *Am. J. Trop. Med. Hyg.* 79: 528-534.
- 437 **Minoli, S. A., and C. R. Lazzari. 2006.** Take-off activity and orientation of triatomines (Heteroptera:
438 Reduviidae) in relation to the presence of artificial lights. *Acta Trop.* 97: 324-330.
- 439 **Noireau, F., and J. P. Dujardin. 2001.** Flight and nutritional status of sylvatic *Triatoma sordida* and
440 *Triatoma guasayana*. *Mem. Inst. Oswaldo Cruz* 96: 385-389.
- 441 **Pacheco-Tucuch, F. S., M. J. Ramirez-Sierra, S. Gourbière, and E. Dumonteil. 2012.** Public street
442 lights increase house infestation by the Chagas disease vector *Triatoma dimidiata*. *PLoS One* 7:
443 e36207.
- 444 **Rodríguez-Planes, L. I., G. M. Vazquez-Prokopec, M. C. Cecere, D. M. Canale, and R. E.**
445 **Gürtler. 2016.** Selective insecticide applications directed against *Triatoma infestans*
446 (Hemiptera: Reduviidae) affected a nontarget secondary vector of Chagas disease, *Triatoma*
447 *garciahesi*. *J. Med. Entomol.* 53: 144-151.
- 448 **Sainz, A. C., L. V. Mauro, E. N. Moriyama, and B. A. García. 2004.** Phylogeny of triatomine
449 vectors of *Trypanosoma cruzi* suggested by mitochondrial DNA sequences. *Genetica* 121: 229-
450 240.
- 451 **Schofield, C. J., M. J. Lehane, P. McEwen, S. S. Catalá, and D. E. Gorla. 1991.** Dispersive flight by
452 *Triatoma sordida*. *Trans. R. Soc. Trop. Med. Hyg.* 85: 676-678.
- 453 **Schofield, C. J., M. J. Lehane, P. McEwen, S. S. Catalá, and D. E. Gorla. 1992.** Dispersive flight by

- 454 *Triatoma infestans* under natural climatic conditions in Argentina. Med. Vet. Entomol. 6: 51-
455 56.
- 456 **Sjögren, R. D., and R. E. Ryckman. 1966.** Epizootiology of *Trypanosoma cruzi* in southwestern
457 North America Part VIII: nocturnal flights of *Triatoma protracta* (Uhler) as indicated by
458 collections at black light traps. J. Med. Entomol. 3: 81-92.
- 459 **Stata Corp. 2017.** Stata Statistical Software. Release 14.2. College Station: Stata Corporation.
- 460 **Stroppa, M. M., M. S. Lagunas, C. S. Carriazo, B. A. Garcia, G. Iraola, Y. Panzera, and N. M.**
461 **Gerez de Burgos. 2013.** Differential expression of glycerol-3-phosphate dehydrogenase isoforms
462 in flight muscles of the Chagas disease vector *Triatoma infestans* (Hemiptera, Reduviidae). Am. J.
463 Trop. Med. Hyg. 88: 1146-1151.
- 464 **Turienzo, P., and O. R. Di Iorio. 2014.** Insects in birds' nests from Argentina. *Pseudoseisura lophotes*
465 Reichenbach, 1853 and *Anumbius annumbi* (Vieillot, 1817) (Aves: Furnariidae), hosts of
466 *Triatoma platensis* Neiva, 1913 (Hemiptera: Reduviidae: Triatominae). Zootaxa 3766: 1-82.
- 467 **Vazquez-Prokopec, G. M., L. A. Ceballos, P. L. Marcet, M. C. Cecere, M. V. Cardinal, U. Kitron,**
468 **and R. E. Gürtler. 2006.** Seasonal variations in active dispersal of natural populations of
469 *Triatoma infestans* in rural north-western Argentina. Med. Vet. Entomol. 20: 273-279.
- 470 **Vazquez-Prokopec, G. M., M. C. Cecere, U. Kitron, and R. E. Gürtler. 2008.** Environmental and
471 demographic factors determining the spatial distribution of *Triatoma guasayana* in peridomestic
472 and semi-sylvatic habitats of rural northwestern Argentina. Med. Vet. Entomol. 22: 273-282.
- 473 **Vezzani, D., N. J. Schweigmann, S. M. Pietrokowsky, and C. Wisnivesky-Colli. 2001.**
474 Characterization of *Triatoma guasayana* biotopes in a hardwood forest of Santiago del Estero,
475 Argentina. Mem. Inst. Oswaldo Cruz 96: 459-466.
- 476 **Wygodzinsky, P., and J. W. Abalos. 1950.** *Triatoma guasayana* Wygodzinsky y Abalos, 1949, con
477 notas sobre especies vecinas (Triatominae, Reduviidae, Hemiptera). An. Inst. Med. Reg. Tucumán
478 3: 53-73.
- 479 **Yeo, M., N. Acosta, M. Llewellyn, H. Sánchez, S. Adamson, G. A. Miles, E. López, N. González, J.**
480 **S. Patterson, M. W. Gaunt, A. R. de Arias, and M. A. Miles. 2005.** Origins of Chagas
481 disease: *Didelphis* species are natural hosts of *Trypanosoma cruzi* I and armadillos hosts of
482 *Trypanosoma cruzi* II, including hybrids. Int J Parasitol. 35: 225-233.

- 483 **Zacharias, C. A., G. B. Pontes, M. G. Lorenzo, and G. Manrique. 2010.** Flight initiation by male
484 *Rhodnius prolixus* is promoted by female odors. *J. Chem. Ecol.* 36: 449-451.
- 485 **Zeledón, R., J. A. Ugalde, and L. A. Paniagua. 2001.** Entomological and ecological aspects of six
486 sylvatic species of triatomines (Hemiptera, Reduviidae) from the collection of the National
487 Biodiversity Institute of Costa Rica, Central America. *Mem. Inst. Oswaldo Cruz* 96: 757-764.
- 488 **zu Dohna, H., M. C. Cecere, R. E. Gürtler, U. Kitron, and J. E. Cohen. 2009.** Spatial re-
489 establishment dynamics of local populations of vectors of Chagas disease. *PLoS Negl. Trop.*
490 *Dis.* 3: e490.
- 491

19

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

494

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

495

496

20

497
498
499
500

Table 2. Concurrent catch of both sexes of *Triatoma infestans*, *Triatoma guasayana* and *Triatoma eratyrisiformis* 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	
<i>Triatoma infestans</i>	Yes	11	24	$P=1.00$
	No	25	365	
<i>Triatoma guasayana</i>	Yes	9	51	$P<0.0001$
	No	15	350	
<i>Triatoma eratyrisiformis</i>	Yes	10	29	$P=0.012$
	No	12	374	

501
502

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"

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

506

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

510

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