Variability of the Susceptibility to Deltamethrin in *Triatoma infestans*: The Female Factor

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ABSTRACT We analyzed the variability of susceptibility to deltamethrin in putatively susceptible *Triatoma infestans* Klug (Hemiptera: Reduviidae), and evaluated the sample size implications on the hypotheses used in the current World Health Organization protocol for the measure of insecticide resistance in Triatominae. Following the protocol, using topical application of deltamethrin to unfed first instar nymphs of *T. infestans*, we found that susceptibility showed significant differences between offspring from different females, a significant association with female age, and significant interaction female × female age. Considering individual female data, three patterns of nymphal mortality were identified: one showed a strong positive relation between nymphal mortality and their mother's age, another showed high mortality with low variability and the third showed intermediate mortality with high variability along female age. The analysis suggests revision of the World Health Organization protocol for resistance detection in Triatominae, not only to take into consideration the sources of variation in susceptibility, but also the effects of sample size in relation to the significance and power probabilities of the test.

KEY WORDS *Triatoma infestans*, insecticide resistance, susceptibility variability, Chagas disease, pyrethroids

Chagas disease, or American trypanosomiasis, is a parasitic disease caused by *Trypanosoma cruzi*. It has been estimated that 10 million people are infected worldwide, mostly in Latin America where Chagas disease is endemic (World Health Organization [WHO] 2010, Coura and Albajar 2010). Because of the absence of a vaccine and the limited efficacy of the currently available parasiticide drugs, the main approaches to control are based on insecticidal control of the insect vectors, house improvement, and health education, together with blood bank screening and treatment of infected children under 15 yr old (Schofield et al. 2006). *Triatoma infestans* Klug (Hemiptera: Reduviidae) is the main vector species within the area of the Gran Chaco, an ecosystem covering around one million square kilometers in the northwest of Argentina, eastern Bolivia, and western Paraguay (Gorla and Noireau 2010).

Since the 1980s, pyrethroid insecticides (particularly deltamethrin) have been the main products used to control domestic Triatominae, because of their efficacy, persistence, and low environmental impact (Zerba et al. 1997, Pinchin et al. 1980, Gürtler et al. 2007, Gorla et al. 2010). During the last decade however, variable levels of pyrethroid resistance in *T. infestans* have been found in parts of Argentina and Bolivia (with resistance rates up to 491) (Picollo et al. 2005, Vassena and Picollo 2003, González Audino et al. 2004, Santo Orihuela et al. 2008, Toloza et al. 2008, Lardeaux et al. 2010, Gemio et al. 2010), with some resistance also to fipronil in Bolivia (Toloza et al. 2008, Germano et al. 2010a). The mechanism of resistance is not yet clear, although current studies suggest that more than one mechanism may have independently evolved in different geographical populations (Toloza et al. 2008, Germano et al. 2010b).

Before the existence of resistance reports in Triatominae, the WHO produced a protocol to evaluate their insecticide susceptibility (WHO 1994). This protocol allowed the realization of standardized studies and led to a first map of the apparent distribution of pyrethroid resistance in *T. infestans* in Argentina (Picollo 2001). The basic rule of the protocol establishes that using a sample of 30 unfed first instar nymphs (N1), a mortality of 0.93 (28 out of 30) or lower should lead to an estimation of the resistance ratio, whereas a mortality of 0.97 (29 out of 30) or higher gives the argument that the nymph sample came from a susceptible population. A sample size of 30 N1 implies it is good enough to make a reliable estimation of the population parameter S (susceptibility) of the *T. infestans* population from where the N1 sample was extracted. Although the variable under study is random, no demographic aspect and/or studies on sources of variability of the susceptibility to pyrethroids in Triato-

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minae, nor an evaluation of the sample size effect, have been reported to our knowledge. Here we explore the variability of pyrethroid susceptibility between first instar nymphs produced by a single adult female, the variability of pyrethroid susceptibility between first instar nymphs produced by different adult females, and the effect of sample size under the probability theory of decision making.

Materials and Methods

Insects. *T. infestans* fifth instar nymphs provided by the breeding facility of the Coordinación Nacional de Control de Vectores (Punilla, Córdoba) were used as the parental group of the experimental nymphs of this study. The fifth instar nymphs were F1 descendants of insects collected in the Departments of San Martín, Belgrano and Capital (San Luis province, central Argentina), from where there were no reports of vector control failure, although a low resistance degree of 3.0 was reported for the nearby areas of Belgrano and Ayacucho Departments (Vassena and Picollo 2003). The insects were bred under controlled temperature and humidity conditions (28° ± 1°C, 50 ± 2%) and a photoperiod of 12:12 h (L:D). Before molting to adults, fifth instar nymphs (N5) were sexed and distributed in 30 labeled groups with one N5 female and three males, to maximize fertilization chance (although we have not been able to count the number of males that effectively copulated with the female) and to carry out the study of the descendants of each individual female. Fifth instar nymphs and adults were weekly fed on chickens. Date of adult emergence and date of initiation of egg production were recorded for each group.

Experimental Design. Eggs deposited by each adult female were collected weekly and stored in jars labeled by female and date. Eggs were collected until around 150 eggs were laid by each female, along a study period of ≈10 wk (variations occurred because some females died before the 10-wk period ended). After egg hatching, N1 between 5 and 7 d old and unfed because eclosions were weighed in batches and transferred to petri dishes with filter paper. Two-thirds of each batch of nymphs was randomly assigned to a treatment, and one-third to the corresponding control group.

All treatment procedures followed the WHO protocol (WHO 1994). The treatment consisted of topical application, using a 10 μl Hamilton syringe, on the dorsal part of the abdomen of a 0.2 μl acetone dilution of deltamethrin((S)-cyano-3-phenoxybenzyl(1R)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate) as active ingredient (a.i.) (Bayer, technical grade 96%), at a concentration of 0.01 mg a.i./ml, which had been previously calculated as the discriminant dose for susceptible *T. infestans* (Vassena and Picollo 2003). Nymphs of the control group were similarly treated with 0.2 μl acetone alone. Mortality of control and treated insects was recorded after 72 h. Insects were considered dead if they were unable to walk from the center to the border of a 7-cm filter paper disc, even when they were mechanically stimulated (WHO 1994). Approximately 100 nymphs descendants per female were treated with deltamethrin, except for three females (10%) that died before the planned number was completed.

Data Analysis. Mortality of the N1 batches was compared using a Kruskal Wallis test. The effect of female, female age and its interaction on N1 mortality was analyzed through a mixed model after heteroscedasticity correction (Jiang 2010). These statistical calculations were carried out with Infostat 2011 (Di Rienzo et al. 2011).

The relationship between the female age and N1 mortality was analyzed using a standard logistic linear regression of the Egret software package (Egret 1999). Average mortality and its variability were calculated for each female and each week, along the studied period of 10 wk. Following the WHO protocol, first instar nymphs had been collected once a week. The number of N1 individuals in each week batch varied according to the egg laying rate of each female. Because batch size was sometimes low, additional comparisons were made grouping nymphs born during 1, 2, 3, and 4 consecutive weeks, to evaluate the effect of sample size on the outcome.

As the evaluation of insecticide susceptibility is based on sampling theory, and mortality is a dichotomous random variable with a probability distribution that can be described by a binomial probability distribution, a power analysis of sample size was carried out, according to the standards recommended by the WHO protocol (WHO 1994). The binomial probability distribution allows the calculation of probability of a random variable × assuming a particular value × from a sample of size n taken from a dichotomous population with $e(X) = P$ and $Var(X) = PQ/n$ (where $Q = 1 - P$). When a susceptibility test is carried out, the implicit hypothesis being tested is that the mortality in the population is greater than or equal to 0.97 (H0) $P \geq 0.97$ (=a pyrethroid susceptible population), against the alternative hypothesis that the mortality is lower than 0.97 (H1) $P < 0.97$). Two possible errors can be made: Type 1 (E1 = rejecting a true H0) and Type 2 (E2 = not rejecting a false H0). The probability of E1 is the significance level of the test and is denoted by α; the probability of E2 is denoted by β and its complement (1-β) is called the power of the test. The regular practice is to assign to α a value of 0.05 (or 0.01) and carry out the statistical calculations accordingly. However, this omits consideration of the power of the test, that in the present case may be more important than the significance level, because erroneously concluding that a sample comes from a susceptible population (when it really comes from a resistant one) has more serious implications than rejecting that the sample comes from a resistant population (when it really comes from a susceptible population). In the latter case (not rejecting a false H0) the WHO protocol suggests carrying out further studies to determine the resistance level, giving additional opportunities to correct possible previous errors. In the former case (accepting a false H0) the protocol

Table 1. Average mortality (min.-max, number of descendant N1, number of wk producing eggs) of treated first instar nymphs of *T. infestans*, descendants of each of the 30 individual females studied, after the 10-wk study period

Female	Average (min.-max, no., wk)	Female	Average (min.-max, no., wk)
1	0.63^{a} (0.40–0.71, 125, 7)	16	0.94^{cdef} (0.80–1.0, 100, 9)
2	0.72^{abc} (0.30–1.0, 100, 8)	17	0.95^{cdef} (0.80–1.0, 100, 10)
3	0.73^{abc} (0.50–1.0, 100, 7)	18	0.95^{cdef} (0.82–1.0, 100, 9)
4	0.76^{abc} (0.55–1.0, 59, 4)	19	0.95^{cdef} (0.89–1.0, 100, 8)
5	0.78^{ab} (0.63–1.0, 100, 0)	20	0.95^{cdef} (0.80–1.0, 100, 7)
6	0.80^{ab} (0.75–0.89, 100, 7)	21	0.95^{cdef} (0.83–1.0, 64, 9)
7	0.87^{bcd} (0.64–1.0, 100, 9)	22	0.97^{cdef} (0.90–1.0, 98, 10)
8	0.87^{bcd} (0.71–1.0, 100, 10)	23	0.98^{def} (0.95–1.0, 100, 7)
9	0.90^{bcde} (0.78–1.0, 100, 7)	24	0.98^{ef} (0.91–1.0, 100, 8)
10	0.91^{bcde} (0.82–1.0, 100, 10)	25	0.99^{ef} (0.90–1.0, 100, 10)
11	0.91^{cdef} (0.67–1.0, 100, 7)	26	1.00^{f} (1.0–1.0, 100, 11)
12	0.93^{cdef} (0.75–1.0, 100, 8)	27	1.00^{f} (1.0–1.0, 100, 10)
13	0.93^{cdef} (0.78–1.0, 100, 8)	28	1.00^{f} (1.0–1.0, 100, 10)
14	0.94^{cdef} (0.75–1.0, 100, 10)	29	1.00^{f} (1.0–1.0, 100, 10)
15	0.94^{cdef} (0.78–1.0, 100, 9)	30	1.00^{f} (1.0–1.0, 100, 10)

Different letters indicate significant differences (Kruskall-Wallis, $P < 0.001$).

does not indicate further studies, so the conclusion has no means of being corrected. Because of the importance of this problem, we carried out a power analysis of the susceptibility to insecticides in *T. infestans.* In the WHO protocol, the H0 is set at $S \geq 0.97$. A power analysis allows calculating the power of a test for a particular value of α and particular values of the alternative hypothesis (H1) and sample size. In the present analysis, we calculated the power value using the binomial probability distribution for sample sizes within the range 10–100 N1 individuals, and values of S between 0.70–0.97.

Results

A total of 4,769 first instar nymphs, average mean weight 1.3 ± 0.2 mg, were produced by the 30 females during the 10 wk period studied. The control group, totaling 1,823 nymphs in 244 batches, showed no mortality during the 3 d established by the WHO protocol. Of the 2,946 treated nymphs, distributed in 242 batches, 2,657 died and 289 survived, giving an overall mortality of 0.90 within the three observed days. The mortality of N1 offspring showed significant difference between females (Table 1; $P < 0.001$, Kruskal Wallis).

Batch size used to calculate N1 mortality ranged from 2 to 36 (minimum - maximum; average = 12.2) in 1-wk groupings, to 25–81 (minimum - maximum; average = 46.6) in 4-wk groupings (Table 2). When N1 mortality was analyzed considering female age, logistic regressions showed significance ($P < 0.001$) for the N1 batch groupings of one and 2 wk (regression coefficients 0.092 and 0.090, respectively, with odds ratios of 1.097 [1.045–1.15 95% CI] and 1.094 [1.038–1.154 95% CI], respectively, but not significant for the three and 4 wk, although only two female age values are included within those groupings). Figure 1 presents the relationship between female age and N1 mortality.

Table 2. Number of first instar nymphs per batch within each female age interval of 1, 2, 3, or 4 wk

Female age consecutive interval	Min. batch size	Max batch size	Average batch size
1-wk	2	36	12.2
2-wk	5	56	22.9
3-wk	12	67	34.6
4-wk	28	81	46.6

The mixed model analyses comparing N1 mortality between all females, N1 mortality against female age, and their interaction, were all highly significant ($P < 0.001$). The significance of the interaction (female × female-age) suggests additional heterogeneity within females and female age, and the variation of N1 mortality within batches from individual females shows three different mortality patterns. One group of females (six out of 30 = 20%) showed a strong positive association between female age and mortality of their N1 descendants, with youngest females (1 wk of age) associated with an N1 mortality of 0.57 ($n = 82$) and oldest (≥8 wk) associated with an N1 mortality of 0.98 ($n = 55$; Fig. 2A). A second group of females (36.7%, 11/30) showed an average mortality of 98.1% with very low variability ($n = 98$, 96 observations ≥0.90 mortality) and no increase in N1 mortality with female age (Fig. 2B). The third group of females

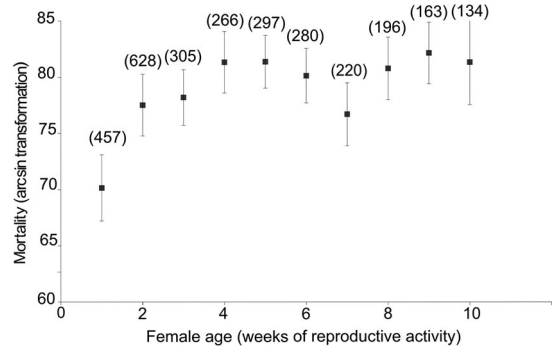

Fig. 1. Relationship between N1 mortality (arcsine transformation) against mother's age (as weeks of reproductive activity). Vertical bars are standard errors. Numbers over each data point are total number of N1 used to estimate mortality for the particular female age.

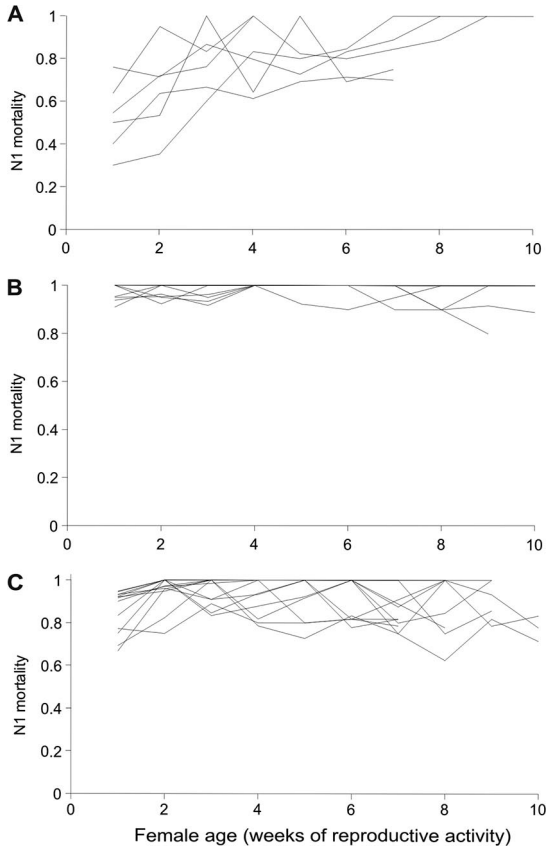

Fig. 2. Three patterns of N1 mortality against *T. infestans* female age (in weeks). (A) Positive relationship in six out of the 30 studied females; (B) no relationship, with low variability in 11 out of the 30 studied females; (C) no relationship with high variability in 13 out of the 30 studied females.

(43.3%, 13 out of 30) also showed no increase of N1 mortality with female age, with an average mortality of 0.91 with high variability; an extreme minimum of 0.62 and first quartile = 0.81 (n = 1,264; Fig. 2C). Among the studied females, 20% (six out of 30) produced N1 of low susceptibility (average mortality 0.64–0.80%, n = 584). Overall, a higher variability of N1 mortality was observed within the offspring of younger females (Fig. 3).

Power Analysis. Under the currently used WHO protocol, the null hypothesis of the susceptibility (S) (test is set at H0) S = 0.97, with a significance level of α = 0.05 and sample size of n = 30. A power analysis of these values shows that for any population parametric value of N1 susceptibility to deltamethrin S $\geq$ 0.82, the test power will be (1-β)$<$ 0.80, that is, $\geq$0.20 of accepting a false H0 (concluding that the first instar nymph sample is susceptible to the insecticide when it is not). The result is presented as power curves, displaying power (1-β) against values of H1 in the range 0.70–0.97 in Fig. 4, for n = 10, 30, 60, and 100.

The lack of consideration of the type 2 error (E2) in the test and the low value of α (to avoid rejection of a true H0) causes the analysis to have low power

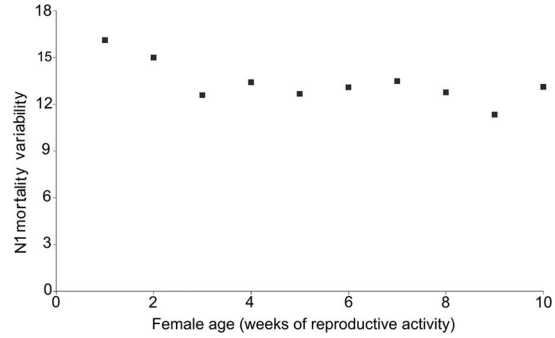

Fig. 3. Relationship between the variability of N1 mortality (arcsine transformation, SD) against female age (as weeks of reproductive activity). Each data point is based on the sample size reported in Fig. 1.

even at relatively high sample sizes. Allowing a higher probability of type 1 error (E1) (rejecting H0 given H0 true) and putting a limit to E2 probability at 0.2, any population with susceptibility average S $\geq$ 0.86 could not be distinguished from S = 0.97 with a sample size of n = 30. The only way of increasing precision without increasing α and β would be to increase the sample size. Figure 4 shows that to distinguish a sample coming from a population with S = 0.97 with any S $>$ 0.90, a sample size of 60 would be needed, and with any S $\geq$ 0.92, a sample of size 100 would be needed.

Discussion

The emergence of insecticide resistance in Triatominae vectors of *T. cruzi* was considered unlikely because of the well known *K*-strategy of their populations and long generation time, contrasting with the *r*-strategy and short generation time of several mosquito species that became resistant to insecticides after a few years of vector control interventions (Gorla 1994, Coleman and Hemingway 2007). In addition, the original operational strategy designed to quickly eliminate all domestic populations from each target area was expected to impede resistance development (Schofield and Dias 1999). The discovery of pyre-

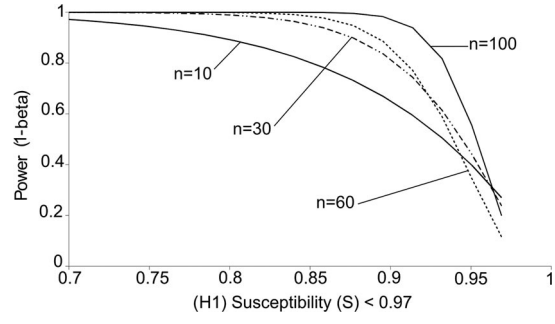

Fig. 4. Power curves for different sample sizes (n) under the null hypothesis (H0) of S (susceptibility to pyrethroid) $\geq$ 0.97, for α = 0.15, against values of the alternative hypothesis H1) S $<$ 0.97.

throid-resistant populations of *T. infestans* in northern Argentina by the end of the 1990s, triggered the quest for other resistant populations in Argentina and neighboring countries.

Several recent studies detected intermediate levels of pyrethroid resistance in *T. infestans* populations from northern Argentina, and highly resistant populations in the Bolivian Chaco (Picollo et al. 2005, Lardeaux et al. 2010, Gemio et al. 2010). Resistance to insecticides in *T. infestans* is still poorly understood, and the cause of resistance emergence remains under discussion. It is not completely clear that the phenomenon appeared as a consequence of mismanagement or overuse of pyrethroid insecticides, as frequently happened in other insect pests (Mota-Sanchez et al. 2008). The genetic variability of the studied populations, associated with their relatively close proximity to the probable center of origin of the species (Panzera et al. 2004, Bargues et al. 2006), raises the possibility of a preadaptative mechanism that produces tolerance to insecticides rather than a resistance phenomenon based on individual selection. The spatially highly structured populations of *T. infestans* at the macro and microgeographic scales (Hernandez et al. 2008, 2011), together with evidence of multiple (and probably independent) resistance mechanisms (Toloza et al. 2008), suggest that the tolerance hypothesis cannot be discarded.

According to the current method for testing resistance to insecticides in Triatominae (WHO 1994), the repeated survival of at least one survivor in two out of three tests (each test with 10 first instar nymphs) using a discriminant insecticide dose, indicates development of resistance and justifies further study. This approach appeared effective for the survey of resistant populations, but the current study shows a number of variability sources of insecticide susceptibility and sampling issues, that should be considered in future revisions of the protocol used for the evaluation of susceptibility to insecticides in Triatominae.

The first result of this study was that the specimens we used, offspring of a colony regularly provided to carry out studies on different aspects of the biology and ecology of *T. infestans*, have less than expected susceptibility to deltamethrin. This finding reinforces the technical request for carrying out regular surveillance of insecticide susceptibility of field collected specimens.

A second result of this study refers to the finding of unexpected sources of variability to insecticide susceptibility in first instar nymphs of *T. infestans.* So far, the variability considered in the analysis of susceptibility to insecticides related with differences between first instar nymphs, and the expectation that with a convenient number of replicates, this variability could be controlled. However, the current study showed that besides the variability between nymphs, variability between and within females is present and can be highly significant. Although statistical tests showed a significant effect of female age, averaging for all females, a closer look at individual female data showed that the detected age effect was caused by the strong association between female age and N1 mortality in 20% of the females, where the younger ones produced nymphs that are less susceptible to deltamethrin and show higher variability in susceptibility, compared with offspring from older females. Although the other 80% of females did not show a strong age N1-mortality association, one group of females (36.7%) showed high mortality and very low variability, whereas the other (43.3%) showed intermediate mortality with high variability.

The results related with sources of variation in the susceptibility to deltamethrin in first instar nymphs have demographic implications in *T. infestans* populations developing under highly seasonal environments, especially with temperature variation. The result of the evaluation of the susceptibility of first instar nymphs could depend on the season the evaluation is carried out. As a higher proportion of young adult females will be present at the beginning of summer than in winter, a lower susceptibility of their first instar nymphs might result (Gorla and Schofield 1989, Gorla 1991).

If a field sample is taken at the beginning of summer, with a high proportion of young adult females, their N1 offspring would be less susceptible, and as N1 mortality from younger females is more variable, a higher sample size would be needed. If the sample is taken at the end of winter, the sample will likely be composed of old females, that would produce N1 more susceptible, and as N1 mortality from older females is less variable, a lower sample size would be needed.

The implications of erroneous decisions from a susceptibility test (susceptible vs. resistant) have different relative weights. As it is more important to avoid the error of identifying a sample as susceptible (when it is resistant) than identifying a sample as resistant (when it is susceptible), the accepted probability for each type of error should be reflected in the design of the susceptibility test. Accepting a higher significance level ($\alpha = 0.20$) than currently used, adopting a power of 0.80 ($\beta = 0.20$) and recommending a sample size of $n = 60$, would establish a stronger scheme for routine susceptibility testing. Given the sources of variability detected in the current study, the protocol should indicate explicitly the need for a repetition of tests based on small samples. Moreover, given the findings of heterogeneous N1 mortality between females, it is highly recommended that not only the selected number of N1 specimens is appropriate, but also that the N1 used for the test are randomly selected from the offspring of a minimum number of five females of similar age or 10 females of different or unknown age.

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References Cited

Bargues, M. D., D. R. Klisiowicz, F. Panzera, F. Noireau, A. Marcilla, R. Perez, M. G. Rojas, J. E. O'Connor, F. Gonzalez-Candelas, C. Galvão, et al. 2006. Origin and phylogeography of the Chagas disease main vector *Triatoma infestans* based on nuclear rDNA sequences and genome size. Infect. Genet. Evol. 6: 46–62.

Coleman, M., and J. Hemingway. 2007. Insecticide resistance monitoring and evaluation in disease transmitting mosquitoes. J. Pestic. Sci. 32: 69–76.

Coura, J. R., and V. P. Albajar. 2010. Chagas disease: a new world challenge. Nature Outlook 465: S6–S7. doi:10.1038/nature09221.

Di Rienzo J. A., F. Casanoves, M. G. Balzarini, L. Gonzalez, M. Tablada, and C. W. Robledo. 2011. InfoStat versión 2011. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. (http://www.infostat.com.ar).

Egret for Windows. 1999. EGRET users manual for Windows, version 2.0.31. Cytel Software Corporation, Seattle, WA.

Gemio, A., N. Romero, M. L. Hernandez, X. Alberto, X. Franz, S. S. Catalá, and D. E. Gorla. 2010. Residual effect of microencapsulated organophosphate insecticides on the mortality of *Triatoma infestans* in the Bolivian Chaco region. Mem. Inst. Oswaldo Cruz. 105: 752–756.

Germano, M. D., G. Roca Acevedo, G. A. Mougabure Cueto, A. C. Toloza, C. V. Vassena, and M. I. Picollo. 2010a. New findings of insecticide resistance in *Triatoma infestans* (Heteroptera: Reduviidae) from the Gran Chaco. J. Med. Entomol. 47: 1077–1081.

Germano, M. D., C. V. Vassena, and M. I Picollo. 2010b. Autosomal inheritance of deltamethrin resistance in field populations of *Triatoma infestans* (Heteroptera: Reduviidae) from Argentina. Pest. Manag. Sci. 66: 705–708.

González Audino, P., C. Vassena, S. Barrios, E. Zerba, and M. I Picollo. 2004. Role of enhanced detoxication in a deltamethrin-resistant population of *Triatoma infestans* (Hemiptera, Reduviidae) from Argentina. Mem. Inst. Oswaldo Cruz. 99: 335–339.

Gorla, D. E. 1991. Recovery of *Triatoma infestans* populations after insecticide application: an experimental field study. Med. Vet. Entomol. 53: 311–324.

Gorla, D. E. 1994. Perspectivas biológicas y ecológicas para el desarrollo de resistencia en Triatominos. Acta Toxicol. Arg. 2: 48–51.

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

Gorla, D. E., and F. Noireau. 2010. Geographic distribution of triatominae vectors in America, pp. 209–231. *In* M. Tybairenc and J. Telleria (eds.), American Trypanosomiasis Chagas disease. Elsevier, España.

Gorla, D. E., C. Ponce, J. P. Dujardin, and C. J. Schofield. 2010. Control strategies against Triatominae, pp. 233–245. *In* M. Tybairenc and J. Telleria (eds.), American Trypanosomiasis Chagas disease. Elsevier, España.

Gürtler, R. E., U. Kitron, M. C. Cecere, E. L. Segura, and J. E. Cohen. 2007. Sustainable vector control and management of Chagas disease in the Gran Chaco, Argentina. Proc. Natl. Acad. Sci. U.S.A. 104: 16194–16199.

Hernández, M. L., L. Abrahan, M. Moreno, D. E. Gorla, and S. Catala. 2008. Phenotypic variability associated to genomic changes in the main vector of Chagas disease in South America. Acta Trop. 106: 60–67.

Hernández, M. L., L. B. Abrahan, J. P. Dujardin, D. E. Gorla, and S. S. Catalá. 2011. Phenotypic variability and population structure of peridomestic *Triatoma infestans* in rural areas of the arid Chaco (Western Argentina): spatial influence of macro- and microhabitats. Vector Borne Zoonotic Dis. 11: 503–513.

Jiang, J. 2010. Large sample techniques for statistics. Springer, New York.

Lardeux, F., S. Depickère, S. Duchon, and T. Chavez. 2010. Insecticide resistance of *Triatoma infestans* (Hemiptera, Reduviidae) vector of Chagas disease in Bolivia. Trop. Med. Int. Health. 15: 1037–1048.

Mota-Sanchez, D., M. E. Whalon, R. M. Hollingworth, and Q. Xue. 2008. Documentation of pesticide resistance in arthropods, pp. 32–39. *In* M. E. Whalon, D. Mota-Sanchez, and R. M. Hollingworth (eds.), Global pesticide resistance in arthropods. Cromwell Press, Trowbridge, United Kingdom.

Panzera, F., J. P. Dujardin, P. Nicolini, M. N. Caraccio, V. Rose, T. Tellez, H. Bermúdez, M. D. Bargues, S. Mas-Coma, J. E. O'Connor, and R. Pérez. 2004. Genomic changes of Chagas disease vector, South America. Emerg. Infect. Dis. 10: 438–446.

Picollo, M. I. 2001. Avances en el monitoreo de resistencia en triatominos y sus necesidades futuras, pp. 13–20. *In* Fundación Mundo Sano (eds.), Serie Enfermedades Transmisibles "Monitoreo de la resistencia a triatominos en América Latina." Mundo Sano, Buenos Aires, Argentina.

Picollo, M. I., C. V. Vassena, P. Santo Orihuela, S. Barrios, M. Zaidemberg, and E. Zerba. 2005. High resistance to pyrethroid insecticides associated with ineffective field treatments in *Triatoma infestans* (Hemiptera: Reduviidae) from Northern Argentina. J. Méd. Entomol. 42: 637–642.

Pinchin, R., A. M. Oliveira Filho, D. M Fanara, and D. Gilbert. 1980. Ensaio de campo para avaliação das probabilidades de uso da decametrina (OMS 1948) no combate a triatomíneos. Rev. Bras. Malariol. Doen. Trop. 32: 36–41.

Santo Orihuela, P. L., C. V. Vassena, E. N. Zerba, and M. I. Picollo. 2008. Relative contribution of monooxygenase and esterase to pyrethroid resistance in *Triatoma infestans* (Hemiptera: Reduviidae) from Argentina and Bolivia. J. Méd. Entomol. 45: 298–306.

Schofield, C. J., and Dias, J.C.P. 1999. The southern cone initiative against Chagas disease. Adv. Parasitol. 42: 1–27.

Schofield, C. J., J. Jannin, and R. Salvatella. 2006. The future of Chagas disease control. Trends Parasitol. 22: 583–588.

Toloza, A. C., M. Germano, G. M. Cueto, C. Vassena, E. Zerba, and M. I. Picollo. 2008. Differential patterns of insecticide resistance in eggs and first instars of *Triatoma infestans* (Hemiptera: Reduviidae) from Argentina and Bolivia. J. Med. Entomol. 45: 421–426.

Vassena, C.V., and M. I. Picollo. 2003. Monitoreo de resistencia a insecticidas en poblaciones de campo de *Triatoma infestans* y *Rhodnius prolixus*, insectos vectores de la Enfermedad de Chagas. RETEL. (http://www.sertox.com.ar/modules.php?name=Content&pa=showpage&pid=104).

(WHO) World Health Organization. 1994. Protocolo de evaluación de efecto insecticida sobre triatominos. Acta Toxicol. Arg. 2: 29–32.

(WHO) World Health Organization. 2010. Chagas disease (American tripanosomiasis). (http://www.who.int/mediacentre/factsheets/fs340/en).

Zerba, E. N., G. Wallace, M. I. Picollo, N. Casabé, S. de Licastro, E. Wood, A. Urvitz, and A. Andres. 1997. Evaluación de la B-cipermetrina para el control de *Triatoma infestans*, vector de la Enfermedad de Chagas. Rev. Panam. Salud Publica. 1: 133–137.

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