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Geographical Variation of Deltamethrin Susceptibility of *Triatoma infestans* (Hemiptera: Reduviidae) in Argentina With Emphasis on a Resistant Focus in the Gran Chaco

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Abstract

Chagas disease is one of the most important parasitic infections in Latin America. The main vector of the protozoan *Trypanosoma cruzi* in America is *Triatoma infestans*, a blood-sucking triatomine bug who is widely distributed in the Gran Chaco ecoregion. Control programs in endemic countries are focused in the elimination of triatomine vectors with pyrethroid insecticides. However, chemical control has failed in the Gran Chaco over the last two decades because of several factors. Previous studies have reported the evolution of different levels of resistance to deltamethrin in *Tri. infestans*. Recently, very high resistance has been found in the central area of the Argentine Gran Chaco. However, the origin and the extension of this remarkably resistant focus remain unknown. The aim of this study was to evaluate the geographical variation of deltamethrin susceptibility of *Tri. infestans* in different endemic provinces of Argentina, with emphasis in the center of the Argentine Gran Chaco ecoregion where this main vector has not been reduced. Populations of Mendoza, San Juan, Santiago del Estero, and Tucumán provinces were all susceptible. Resistant populations were only detected in the province of Chaco, where a mosaic resistant focus was described at the Güemes Department. It was characterized into three pyrethroid resistance categories: susceptible, low, and highly resistant populations. We found the populations with the highest resistance levels to deltamethrin, with resistant ratios over 1000.

Key words: *Triatoma infestans*, Gran Chaco, pyrethroid resistance, toxicological heterogeneity

Chagas disease is one of the most important parasitic infections in Latin America. Around 15–16 million people are estimated to be infected with *Trypanosoma cruzi* and 75–90 million are exposed to infection (Rodrigues-Coura and Dias 2009). The main vector of *T. cruzi* in the southern cone is *Triatoma infestans* (Klug 1834) (Hemiptera: Reduviidae), a blood-sucking triatomine bug who exhibits high adaptation to human habitations and peridomestic structures housing domestic animals (Zeledón and Rabinovich 1981). *Triatoma infestans* is widely distributed in the Gran Chaco, an ecoregion covering around one million square kilometers in northern Argentina, eastern Bolivia, and western Paraguay (Gorla and Noireau 2010) where prevalence of infestation remains high.

On a public health scale, the trypanosomiasis is difficult to treat and vaccines are unavailable. Thus, control programs in endemic countries rely primarily on screening of blood donors to reduce the risk of transfusional transmission, maternal screening for

infection, and elimination of the domestic triatomine vectors (WHO 2006).

Pyrethroid insecticides were effective in the elimination of intradomiciliary populations of *Tri. infestans*, and less effective in peridomestic ecotopes housing domestic animals (Gürtler et al. 2004, Zerba 1999). However, in the Gran Chaco ecoregion, the presence of intradomiciliary triatomines after spraying with pyrethroids has frequently been reported (Vazquez-Prokopec et al. 2009). The unsatisfactory control may be explained by several biological, genetic, and environmental causes, and by the evolution of insecticide resistance (Gürtler 2009, Gürtler et al. 2004, Picollo et al. 2005, Vazquez-Prokopec et al. 2009).

Resistance is a population process, which is a consequence of two major factors: the presence of individuals with a heritable capacity to withstand the insecticide and the selection pressure through the insecticide (Tabashnik 1989). The emergence of resistant individuals in a population is very difficult to predict, thus it is

important to detect the resistance at the lowest level such as possible to interrupt the selection process in the endemic area (Mougabure-Cueto and Picollo 2015).

Several studies have reported the evolution of different levels of resistance to pyrethroids in *Tri. infestans* (Germano et al. 2010, Picollo et al. 2005, Toloza et al. 2008), that were classified in different profiles and mechanisms (Germano et al. 2012, Mougabure-Cueto and Picollo 2015) that were found within the geographic distribution of the species. Recently, very high resistance to deltamethrin has been found in an area of Güemes Department (Chaco province of Argentina), in localities such as El Malá (Carvajal et al. 2012), La Esperanza (Germano et al. 2013, 2014) and El Juramento (Sierra et al. 2016). However, the origin and the extension of this highly resistant focus remain unknown. Thus, resistance to deltamethrin in *Tri. infestans* is a complex problem mainly in the Gran Chaco where this main vector has not been reduced and is considered as the last main barrier to be conquered (Gürtler 2009). The aim of this study was to evaluate the geographical distribution of deltamethrin susceptibility of *Tri. infestans* in different endemic provinces of Argentina, with emphasis in the area of high resistance from Güemes Department in the Gran Chaco ecoregion.

Materials and Methods

Insects Sampling and Rearing.

Field *Tri. infestans* were collected in 55 localities of different Argentine Departments of Mendoza, San Juan, Tucumán, Santiago del Estero, and Chaco provinces (Fig. 1; Table 1). The collection was performed through active searches in intradomicile and peridomicile by technicians from the National Chagas Program (NCP) of Argentina. The sampling technique follows the standards established by the NCP for resistance monitoring under which an optimum number of 15 males and 15 females coming from the maximum possible number of homes are captured. Once insects were collected and transported to our laboratory, they were classified by locality and they were raised. After the hatching of the eggs (F1), first instar nymphs were employed in the several analyses. The Stariolo strain was used as the *Tri. infestans* susceptible reference that was obtained from descendants of insects provided by the Vectors Reference Center from NCP (Punilla, Córdoba, Argentina). This control strain has never been in contact with insecticide and was characterized biochemical and genetically by Roca-Acevedo et al. (2015). Field and reference population insects were raised at the laboratory under controlled temperature ($28 \pm 1^\circ\text{C}$), humidity (50–70%), and a photoperiod of 12:12 (L:D) h. A pigeon was weekly provided as a blood meal source (WHO 1994).

Chemicals

The insecticides employed were of technical-grade deltamethrin (99% purity) and fenitrothion (98% purity) and were provided by Dr. Ehrenstorfer (Augsburg, Germany). Analytical grade acetone used for dilutions was purchased from Merck (Buenos Aires, Argentina).

Bioassays

First Instar Nymphs. Test to determinate deltamethrin susceptibility was performed by topical application on *Tri. infestans* first instar nymphs (5–7-d-old, mean weight 1.3 ± 0.2 mg) starved since eclosion (WHO 1994). Each insect was treated with 0.2 μl of the insecticide diluted in acetone on the dorsal abdomen, using a 10- μl Hamilton syringe (Hamilton, Reno, NV) provided with a repeating dispenser (Hamilton PB-600-1). To calculate the LD values at

$P < 0.05$, a minimum of 30 insects per concentration was required (Robertson et al. 2007). Ten insects were used for each replicate, and each dose was replicated at least three times in order to diminish the risk of obtain biased results. Control groups received only pure acetone. The dose that causes 99% mortality of individuals of the susceptible strain (DL₉₉) was used as discriminating dose (DD) and was the initial tested dose. At DD, the usual threshold for detecting resistance is 80% mortality (Brown and Pal 1973); therefore, with a single-dose assay it is possible to detect the presence of resistant individuals from a field population. The DD used for deltamethrin was of 2 ng per insect (i.e., a concentration of 0.01 mg of deltamethrin/ml of acetic solution) (Picollo et al. 2005). For those resistant populations, at least four doses were applied in a range that produced between 10 and 90% of mortality. Final concentrations ranged from 0.0001 to 10 mg/ml for deltamethrin. After exposition, insects were kept at the previously mentioned laboratory conditions for 24 h, and then mortality was evaluated. Criterion for mortality was the inability to walk from the center to the border of a circular 11-cm diameter filter paper. Only those nymphs that were able to reach the filter paper border, with or without mechanical stimulation with forceps, were considered alive (WHO 1994). For those populations that showed to be deltamethrin resistant, a fenitrothion DD of 100 ng per insect (0.5 mg/ml) was tested.

Eggs. Deltamethrin susceptibility was determined on eggs of later embryonic development (11–12-d-old). The external morphological characteristics of the selected eggs were orange with dark eyespots (Picollo de Villar et al. 1979). Groups of 10 eggs per concentration were fixed to a microscope slide by a double-sided tape. Then, each egg was treated by topical application onto the operculum with 0.2 μl of the insecticide diluted in acetone, by using a 10- μl Hamilton syringe (Picollo et al. 1976). Each group of eggs received the DD of 20 ng of deltamethrin per egg (0.1 mg/ml). Controls received acetone. After topical application, eggs were incubated in a rearing cabinet (FOC-225E, Velp Scientifica, Milan, Italy) at $28 \pm 1^\circ\text{C}$, 50%RH, and a photoperiod of 12:12 (L:D) h. Mortality was evaluated 10 d after treatment to determinate the number of eggs that failed to hatch. This period was used to check whether any delayed hatching took place in the control (Toloza et al. 2008).

Statistical Analysis

Data were analyzed using the POLO PLUS Software (LeOra 2007). Mortality data were corrected using Abbott's formula implemented in the POLO PLUS package. Dose-mortality data for first instar nymphs of each *Tri. infestans* population were subjected to probit analysis (Litchfield and Wilcoxon 1949) to estimate the lethal dose (ng/insect) required to kill 50% of treated individuals (LD₅₀). Resistance ratios (RR_{50s}) and 95% confidence limits (CIs) were calculated as described by Robertson et al. (2007). Field populations were considered resistant when RR_{50s} were significantly different from 1 (i.e., when the 95% CI of the RR₅₀ did not include the number one). RR₅₀ < 10 was considered as low resistance, $10 < \text{RR}_{50} < 100$ was considered as medium resistance, and RR₅₀ > 100 was considered as high resistance (Germano et al. 2012). When it was possible, the resistant ratios affecting the 90% of the exposed subjects (RR₉₀) were calculated following the methodology described above.

Results

Bioassay performed on first instar showed that most of the 55 (78%) field populations of the endemic area of *Tri. infestans* were

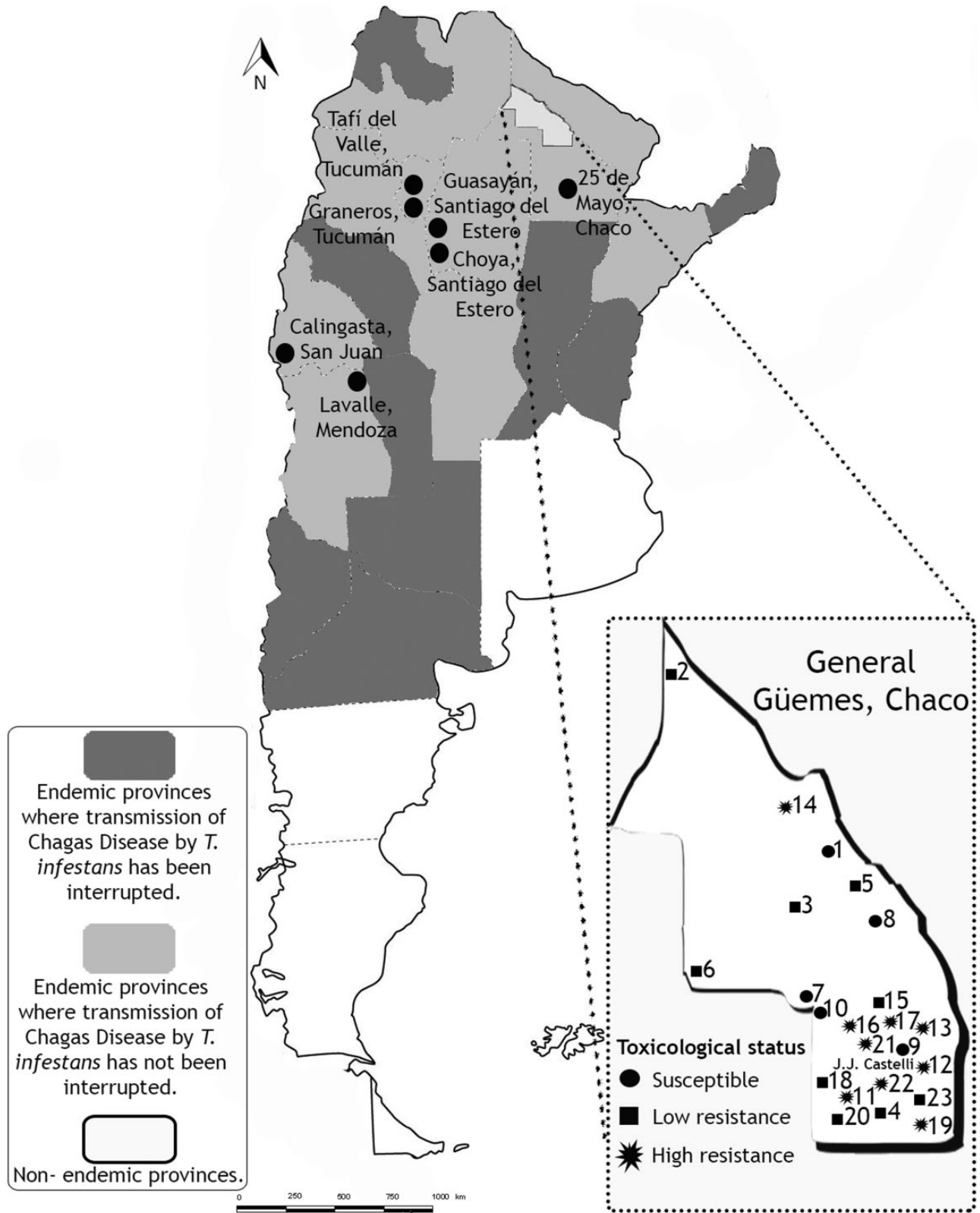


Fig. 1. Geographical distribution of the collected insects. Susceptible departments: Lavelle (Mendoza), Calingasta (San Juan), Choya (Santiago del Estero), Guasayán (Santiago del Estero), Graneros (Tucumán), Tafi del Valle (Tucumán), 25 de Mayo (Chaco). Localities from the resistant focus of Güemes Department (Chaco): 1. Los Placeres, 2. Palma Sola, 3. Los Pereyras, 4. Pozo Colorado, 5. El Maulle, 6. Palo Marcado, 7. Los Quirquinchos, 8. 4 de Febrero, 9. Bella Vista, 10. El Techat, 11. Pampa Almirón, 12. Campo Alto, 13. El Nandú, 14. La Rinconada, 15. El Zanjón, 16. Pampa Argentina, 17. El Juramento, 18. Colonia Castelli, 19. El Asustado, 20. Zaparinqui, 21. El Malá, 22. La Esperanza, 23. La Gerónima.

Table 1. Deltamethrin susceptibility in field populations of *Tri. infestans* in different endemic provinces of Argentina

Province	Department	Site of collection	% Mortality with diagnostic assay (<i>n</i> tested insects)	Deltamethrin susceptibility
Mendoza	Lavalle	Asunción	94 (16)	Susceptible
	Lavalle	San Pedro	100 (10)	Susceptible
	Lavalle	Nueva California	100 (6)	Susceptible
	Lavalle	San José	100 (5)	Susceptible
	Lavalle	California	100 (10)	Susceptible
	Lavalle	El Forzudo	100 (24)	Susceptible
	Lavalle	El Retamo	95 (19)	Susceptible
San Juan	Calingasta	Sorogayense	90 (41)	Susceptible
	Calingasta	La Isla	81 (27)	Susceptible
	Calingasta	Hilario	100 (34)	Susceptible
Sgo. del Estero	Choya	La Represa	96 (23)	Susceptible
	Choya	Pozo del Campo	82 (33)	Susceptible
	Choya	San Justo	91 (44)	Susceptible
	Choya	Balde Pozo	93 (14)	Susceptible
	Choya	Las Peñas	100 (9)	Susceptible
	Choya	San Pedro	100 (30)	Susceptible
	Guasayán	Famatina	81 (21)	Susceptible
	Guasayán	Pampa Pozo	100 (15)	Susceptible
	Guasayán	El Symbol	89 (36)	Susceptible
	Guasayán	Las Chacras	95 (40)	Susceptible
Tucumán	Guasayán	El Galpón	93 (41)	Susceptible
	Graneros	Palo seco	80 (85)	Susceptible
	Graneros	La Barranca	94 (68)	Susceptible
	Graneros	El Simbol	96 (25)	Susceptible
	Graneros	Los Chañarcitos	80 (46)	Susceptible
	Graneros	Los Árboles Grandes	80 (61)	Susceptible
	Tafí del Valle	Tafí del Valle	100 (31)	Susceptible
Chaco	Güemes	Pampa Argentina	3 (66)	Resistant
	Güemes	Pampa Almirón	13 (16)	Resistant
	Güemes	El Ñandú	13 (40)	Resistant
	Güemes	Campo Alto	2 (41)	Resistant
	Güemes	El Asustado	0 (31)	Resistant
	Güemes	La Rinconada	0 (30)	Resistant
	Güemes	El Juramento ^a	5 (41)	Resistant
	Güemes	El Malá ^b	0 (30)	Resistant
	Güemes	La Esperanza ^c	19 (61)	Resistant
	Güemes	El Maulle	90 (40)	Susceptible
	Güemes	Los Pereyras	93 (40)	Susceptible
	Güemes	Zaparinqui	100 (30)	Susceptible
	Güemes	Pozo Colorado	83 (30)	Susceptible
	Güemes	El Zanjón	77 (82)	Resistant
	Güemes	Palma Sola	77 (31)	Resistant
	Güemes	Palo Marcado	92 (50)	Susceptible
	Güemes	Colonia Castelli	84 (70)	Susceptible
	Güemes	La Gerónima ^a	63 (106)	Resistant
	Güemes	Bella Vista	88 (41)	Susceptible
	Güemes	4 de febrero	100 (30)	Susceptible
	Güemes	Los Quirquinchos	82 (42)	Susceptible
	Güemes	Los Placeres	100 (30)	Susceptible
	Güemes	El Techat	92 (77)	Susceptible
25 de Mayo	Lote 40	96 (81)	Susceptible	
25 de Mayo	Colonia Waltieri	100 (46)	Susceptible	
25 de mayo	Lote 39	100 (97)	Susceptible	
25 de mayo	El Milagro	97 (35)	Susceptible	
25 de mayo	La Tambora	94 (31)	Susceptible	

Data from ^aSierra et al. (2016), ^bCarvajal et al. (2012), ^cGermano et al. (2014).

susceptible to deltamethrin and exhibited mortality higher than 80% at discriminant dose (Table 1). Populations of Mendoza, San Juan, Santiago del Estero, and Tucumán provinces were all susceptible. Resistant populations were located only in the province of

Chaco. In this endemic province, mortality rates were generally <80% and in some localities was 0 (Fig. 1). Therefore, RR_{50S} were calculated and some of the populations classified as susceptible in Table 1 were reclassified as low resistant in Table 2 (El Maulle,

Los Pereyras, Zaparínqui, Pozo Colorado, Palo Marcado, and Colonia Castelli). We found highly variable levels of susceptibility to deltamethrin in Güemes Department populations: 23% were susceptible, 41% showed low resistance, and 36% possessed high resistance. The DL_{50} s of the first instar nymphs within this area ranged from 0.038 to 1,664 nanograms per insect (ng/i).

We have detected very high levels of resistance, as was evidenced by large resistance ratios. In some populations like Pampa Argentina, Campo Alto, El Asustado, and La Rinconada, the mortality at the highest dose applied (10 mg/ml) was <50% and their LD_{50} values were >200. Since it was not possible to obtain a finite estimation, the RR_{50} s were considered to be >1,000.

Eggs treated with deltamethrin showed the same toxicological status that first instars nymphs in each population. All studied populations at the resistant focus were susceptible to fenitrothion, the only approved alternative insecticide to pyrethroids used by the NCP at the field control (Table 2).

Discussion

This research evaluated the geographical variations of deltamethrin susceptibility of *Tri. infestans* in different endemic provinces of Argentina. We found that most of the evaluated populations were susceptible, with the exception of a complex toxicological pattern found in the Gran Chaco ecoregion at Güemes Department. Fortunately, 43 (78%) of the 55 studied localities were susceptible, indicating that deltamethrin is a viable tool used by the Chagas disease control programs of the endemic zones.

The first reports of resistance to deltamethrin were found in *Tri. infestans* populations from Salvador Mazza (northern Argentina) and Yacuiba (southern Bolivia) (Picollo et al. 2005, Toloza et al. 2008). These studies reported that the resistant area was restricted to the Argentinian-Bolivian border, because field populations to the south of S. Mazza-Yacuiba were susceptible or slightly resistant to deltamethrin. Later, Germano et al. (2010) studied several localities from Argentina and Bolivia and reported new findings of insecticide resistance in populations northwards and southward of the Argentinian-Bolivian border with RRs of 500. This indicated the presence of a greater extension area than previously expected. Similarly, Lardeux et al. (2010) found that 28% of the populations were susceptible in Bolivia and proposed that resistance to deltamethrin was widespread throughout the country, with resistance levels ranging from 6 to 491. Our results indicated that the extension of the insecticide resistance in Argentina is geographically restricted to populations from certain zones of the Gran Chaco.

Although insecticide resistance in triatomines is a serious problem, in this study we found that it is restricted to the central area of the Argentine Gran Chaco ecoregion, the main place where the control and elimination of *Tri. infestans* has failed. In the last 20 years, Gran Chaco region is characterized by the discontinuity and heterogeneity of the chemical control actions against triatomines with lack or few interventions (Mougabure-Cueto and Picollo 2015). Specifically, we found the highest resistant zone near the urban city of Castelli (Chaco province). This complex pattern was characterized by three categories: susceptible, low, and high resistant populations. Lardeux et al. (2010) proposed that the heterogeneity in the resistance levels to deltamethrin is likely to originate from non-homogenous insecticide pressure. In fact, the frequencies and the insecticide doses might vary among villages or among intra and peridomestic environments. In addition, insecticide degradation by environmental variables is more likely to occur in the peridomestic

structures and it could promote a different selection in comparison to the intradomestic environment (Gürtler et al. 2004). Thus, selection of individuals exposed to sublethal doses (e.g., in peridomestic) and lethal doses (e.g., in domicile) would lead to the evolution of heterogeneous toxicological profiles (Gressel 2011). Germano et al. (2012) showed important differences in resistance status at the microgeographical level in La Esperanza (Chaco province), suggesting a high level of population structure in *Tri. infestans* on which the insecticide exerts its selective pressure. Previous studies demonstrated that *Tri. infestans* populations are genetically highly structured (Pérez de Rosas et al. 2007, Pizarro et al. 2008, Segura et al. 2009), showing rich genetic variability through its distribution range (Piccinali et al. 2009). Thus, the heterogeneous insecticide application and high-population structure in *Tri. infestans* might explain the heterogeneity of toxicological phenotypes. This can partially account for the observed differences in resistance patterns among very close population at the Güemes Department.

The presence of populations with natural low susceptibility (i.e., tolerance to deltamethrin) in the center of Gran Chaco could not be discarded. Several authors studied several wild populations of *Tri. infestans* from Bolivia and found different susceptibility to deltamethrin. These populations were collected from a sylvatic habitat without chemical control interventions and it seems very unlikely that they were exposed to any sort of insecticide (Bustamante-Gomez et al. 2014, Depickère et al. 2012, Roca-Acevedo et al. 2011). Thus, wild *Tri. infestans* from different geographic areas of this country seems to have different degrees of natural tolerance to deltamethrin. Mougabure-Cueto and Picollo (2015) stated that the discontinuity of the control actions in vast areas of Gran Chaco leads to speculate about the presence of insecticide tolerant populations, and proposed the hypothesis that some environmental variables of the Chaco ecoregion could select individuals with high tolerance without the presence of the insecticide. More research is needed to identify if there are some environmental variables that could be implicated in the promotion of this natural tolerance to insecticides in different populations within the resistant focus.

Finally, it should be noted that the persistence of vector-borne transmission of *T. cruzi* could be related to factors other than entomo-toxicological factors (including resistance evolution). These factors are as following: discontinuity in space and time of the chemical control interventions, high density of triatomines in peridomestic structures housing domestic animals, unavailability of the insecticide, re-infestation/colonization events from nearby rural areas, or sylvatic focus (Gürtler et al. 2007, Porcasi et al. 2006, Vazquez-Prokopec et al. 2009).

To the best of our knowledge, we found the populations with the highest resistance levels to deltamethrin, with RR values exceeding 1000. These high levels of resistance might be determined (at least as part) by the alterations in the site of action of the insecticides, the voltage-sensitive sodium channels, as was demonstrated in several insect species of economic and sanitary importance (Soderlund and Knipple 2008). In addition, Zhu et al. (2010) investigated the distribution and extent of the reduced neuronal sensitivity to pyrethroids and DDT, termed “knockdown resistance” (*Kdr*), in bed bug populations across the United States and found that they were the main reason of resistance. They found that very few populations were resistant to deltamethrin through increased activity of detoxifying enzymes like monooxidases P_{450} . Recently, two point mutations in the sodium channel associated to pyrethroid resistance, L1014F and L925 (in the numbering of the house fly amino acid sequence of the sodium channel) were described in two resistant populations of *Tri. infestans* (Capriotti et al. 2014, Fabro et al. 2012). Some studies in

Table 2. Toxicological effects of deltamethrin and fenitrothion on field populations of *Tri. infestans* from Güemes, Chaco

Population (latitude, longitude)	First-instar nymphs					Eggs		
	Deltamethrin					Fenitrothion		
	<i>n</i>	Slope ± SE	χ^2	LD50 (ng/f) (95%CI)	RR50 (95%CI)	Toxicological status	% Mortality with diagnostic assay (<i>n</i> tested insects)	% Mortality with diagnostic assay (<i>n</i> tested eggs)
Reference (Stariolo)	171	1.41 ± 0.35	11.31	0.18 (0.03–0.35)	–	Susceptible	100 (30)	100 (0)
El Juramento (25°54'S, 60°24'W) ^a	227	0.53 ± 0.09	3.34	1664 (676–6590)	>2000	High resistant	100 (40)	–
El Malá (25°56'S, 60°27'W) ^b	90	1.0 ± 0.4	15.54	134 (100–182)	1031 (909.1–1213.3)	High resistant	93 (42)	0 (90)
La Esperanza ^c (26°3'S, 60°27'W)	224	0.64 ± 0.12	28.63	30.32 (10.21–79.71)	233.42 (116.78–466.57)	High resistant	100 (41)	–
Pampa Argentina (25°53'59"S, 60°29'36"W)	251	7.72	2.20	> 200	> 1000	High resistant	100 (30)	6.5 (31)
El Nándú (25°56'54"S, 60°19'59"W)	251	0.96 ± 0.23	18.95	115.2 (49.6–453)	500 (200–1000)	High resistant	100 (30)	0 (30)
Campo Alto (26°09'S, 60°14'25"W)	190	1.78 ± 0.81	9.61	> 200	> 1000	High resistant	100 (17)	20 (30)
El Asustado (26°5'10"S, 60°18'22"W)	255	1.11 ± 0.74	15.48	> 200	> 1000	High resistant	100 (32)	2 (10)
La Rinconada (24°55'14"S, 61°24'15"W)	198	0.60 ± 0.19	68.59	> 200	> 1000	High resistant	100 (30)	0 (32)
La Gerónima (26°4'S, 60°16'W) ^a	356	0.92 ± 0.12	13.82	0.71 (0.27–1.57)	3.89 (1.63–9.26)	Low resistant	98 (58)	–
El Maulle (25°27'42"S, 60°55'10"W)	210	2.20 ± 0.33	18.21	0.87 (0.58–1.27)	4.72 (2.11–10.63)	Low resistant	96.7 (30)	–
Los Pereyras (25°28'55"S, 61°11'12"W)	302	5.39 ± 1.06	37.00	1.08 (0.82–1.26)	5.68 (2.72–12.66)	Low resistant	93.3 (30)	–
Zaparinqui (26°3'53"S, 60°34'31"W)	120	2.31 ± 0.43	7.22	0.61 (0.36–0.98)	3.29 (1.43–7.52)	Low resistant	100 (31)	–
Pozo Colorado (26°3'59"S, 60°30'29"W)	110	2.58 ± 0.80	2.07	0.96 (0.41–1.35)	5.21 (2.16–12.50)	Low resistant	100 (30)	93 (30)
El Zanjón (25°43'16"S, 60°43'54"W)	370	2.05 ± 0.25	22.72	0.74 (0.57–0.92)	4.03 (1.81–8.93)	Low resistant	100 (30)	100 (30)
Palma Sola (24°14'30"S, 62°16'50"W)	222	1.88 ± 0.34	17.37	0.77 (0.46–1.13)	4.18 (1.81–9.62)	Low resistant	100 (30)	70 (30)
Palo Marcado (24°37'4"S, 62°6'30"W)	384	1.80 ± 0.17	187.66	0.67 (0.37–30)	3.97 (1.78–8.77)	Low resistant	90 (30)	100 (30)
Colonia Castelli (26°0'38"S, 60°39'18"W)	179	2.08 ± 0.34	18.34	0.56 (0.31–85)	3.06 (1.31–7.14)	Low resistant	100 (30)	87 (31)
4 de febrero (25°34'58"S, 60°47'52"W)	118	1.07 ± 0.27	2.45	0.04 (0.02–0.07)	0.19 (0.10–0.51)	Susceptible	–	25 (20)
Los Quirquinchos (25°42'1"S, 60°55'1"W)	155	0.50 ± 0.11	2.30	0.04 (0.004–0.12)	0.20 (0.04–1.05)	Susceptible	–	–
Los Placeres (25°18'7"S, 61°10'56"W)	140	1.010 ± 0.21	1.09	0.04 (0.02–0.07)	0.21 (0.10–0.53)	Susceptible	–	–
Bella Vista (25°57'15"S, 60°22'34"W)	251	1.07 ± 0.18	12.87	0.37 (0.20–0.60)	2.02 (0.80–5.10)	Susceptible	–	–
El Techar (25°42'44"S, 60°53'59"W)	168	2.12 ± 0.32	14.48	0.44 (0.24–0.68)	2.38 (1.01–5.62)	Susceptible	–	–

Data from ^aSierra et al. (2016), ^bCarvajal et al. (2012), ^cGermano et al. (2014). Pampa Almirón population was not analyzed because of the lack of biological material. *n* = number of insects used in the bioassay, SE = standard error, ng/f = nanograms per insect, 95%CI = 95% confidence interval, RR = resistant ratio. In those populations where the mortality at the highest insecticide used dose was <50%, we considered the dose where the slope began to flatten (DL50 ~1 mg/ml ≡ 200 ng/f) to inform as "DL50>200" and the RR values were considered as >1000. NA = not available information (in those populations where it was not possible estimate RR90).

mosquitoes and head lice proposed a strong causal relationship between *Kdr* genotype and susceptibility to pyrethroids (Reimer et al. 2008, Toloza et al. 2014). This allowed the authors to propose that *Kdr* is closely associated with resistance and serves as an effective and practical marker in monitoring actions. This association could also be present in *Tri. infestans* and could explain the presence of populations with high levels of resistance determined by high frequencies of the mutations L1014F or L925S. On the other side, those populations with low levels of pyrethroid resistance might be due to an increased metabolism and decreased penetration.

Concerning eggs, we found that those populations exposed to deltamethrin showed the same toxicological status that those exhibited by first instar nymphs. These results are consistent with Toloza et al. (2008), who demonstrated that eggs from the Argentine Salvador Mazza population were as resistant to deltamethrin as the nymphs, suggesting that the similar levels of resistance found for eggs and first instar nymphs could be based on the expression of similar mechanisms of resistance in both stages. Later, Germano et al. (2012) found that the Bolivian population of Entre Ríos exhibited nymphs with high resistance to deltamethrin, but their eggs showed medium resistance levels to the insecticide. This could imply that the expression of insecticide resistance in eggs varies among Argentinian and Bolivian populations, and pyrethroid resistance diagnosed in first instar nymph is not always indicative of resistance at the egg stage. In addition, Amelotti et al. (2011) showed that females within an age range can produce individuals with different toxicological phenotype and this could explain at least at part the differences in susceptibilities between groups of insects (i.e., populations, colonies, stages, etc.). In order to increase the reliability of the obtained results, this study followed the recommendation that individuals used in bioassays are randomly selected from the offspring of a minimum number of 10 females of unknown age.

All studied resistant populations were susceptible to fenitrothion. The only insecticide formulations approved for use in the field control of *Tri. infestans* in Argentina are those containing the pyrethroid deltamethrin or the organophosphates fenitrothion and malathion (Carvajal et al. 2012). Considering toxicological risk and efficacy, quality of the formulations and acceptability by residents of endemic areas, the rotation between deltamethrin and fenitrothion is not recommended (Mougabure-Cueto and Picollo 2015).

Our findings do not necessary imply that there are no other resistant foci in the studied endemic area. Further research is needed to have a better understanding about *Tri. infestans* deltamethrin susceptibility. Toxicological monitoring in the species of epidemiological importance followed by genetic and evolutionary studies of the resistance is a priority issue since this might give a complete picture of the status susceptibility/resistance in the Chagas' vectors. The emergence of insecticide resistance emphasizes the need of incorporating other tools for integrated Chagas disease control in the Gran Chaco ecoregion.

Housing improvement, environmental and host management, and community development are also key elements to obtain a sustainable vector control strategy which reduce the incidence of the endemic disease in this problematic area.

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