Research Article

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Autosomal inheritance of deltamethrin resistance in field populations of *Triatoma infestans* (Heteroptera: Reduviidae) from Argentina

Mónica D Germano,[a]* Claudia V Vassena[a,b] and María I Picollo[a]

Abstract

BACKGROUND: *Triatoma infestans* (Klug) is the major Chagas disease vector in the Southern Cone area of South America, and its chemical control is based on the use of pyrethroid insecticides. Resistance to deltamethrin in Salta Province, Argentina, has been detected in field populations since 2002, causing the failure of vector control campaigns in this disease-endemic area. The inheritance of deltamethrin resistance in *T. infestans* was evaluated through reciprocal crosses conducted between resistant and susceptible insects.

RESULTS: The response of the reciprocally mated insects' progeny to deltamethrin was intermediate between the highly resistant and the susceptible parent colonies. Lack of significant differences between the LD_{50} and resistance ratios of the reciprocally mated insects indicated no sex linkage on this trait.

CONCLUSION: Bioassay results, in addition to degree of dominance calculations, suggest that the resistance to deltamethrin in *T. infestans* is controlled by semi-dominant, autosomally inherited factors.

Keywords: *Triatoma infestans*; resistance; pyrethroid; deltamethrin; inheritance

1 INTRODUCTION

Chagas disease is a public health concern in most Latin American countries, and its prevention is based on vector insect control. *Triatoma infestans* (Klug) is the major disease vector in the Southern Cone area of South America. The chemical control of this species is mainly based on the use of insecticides, particularly pyrethroids.[1,2] However, in the last decade, different levels of pyrethroid resistance have been detected in an area ranging from northern Argentina to central Bolivia.[3–5]

Because pesticide resistance is a genetic phenomenon, a complete understanding of resistance requires knowledge of how it is inherited.[6] The inheritance of insecticide resistance has been extensively studied in Diptera and Lepidoptera, although little is known with respect to inheritance of resistance to pyrethroids.[7] Inheritance of permethrin resistance has been reported to be autosomal and incompletely recessive in *Plutella xylostella* (L.),[8,9] and incompletely dominant in *Pectinophora gossypiella* (S.).[10] These studies suggest that the manner in which pyrethroid resistance is inherited in lepidopteran pests may vary depending on the species. To date, no studies of resistance inheritance have been reported for Chagas disease vectors.

The mode of inheritance of deltamethrin resistance was studied in recently collected field populations of *T. infestans* from Salta, Argentina, on which baseline toxicity studies had been conducted.[3–5] The present paper reports on the results of experiments to characterise the inheritance of pyrethroid resistance in *T. infestans* from this area, and discusses implications for resistance management.

2 METHODS AND MATERIALS

2.1 Insect sampling and rearing

Deltamethrin-resistant insects (R) were collected from the area of Salvador Mazza (22° 5′ S, 63° 7′ W; northern Salta, Argentina), where high resistance ratios had been the cause of ineffective field treatments since 2002.[3] Susceptible insects (S) were collected from a non-treated area in east Salta, Santa Rosa (23° 54′ S, 63° 11′ W) (Fig. 1). In both cases, individuals were captured with the use of manual forceps from a single house structure, with the aim of decreasing the heterogeneity expected for field populations. A laboratory strain (CIPEIN), which had been reared at the laboratory

* *Correspondence to: Mónica D Germano, Centro de Investigaciones de Plagas e Insecticidas (CIPEIN, CITEFA-CONICET), Juan Bautista de la Salle 4397 (B1603ALO), Villa Martelli, Provincia de Buenos Aires, Argentina. E-mail: mgermano@citefa.gov.ar*

a *Centro de Investigaciones de Plagas e Insecticidas (CIPEIN, CITEFA-CONICET), Villa Martelli, Provincia de Buenos Aires, Argentina*

b *Carrera de Posgrado del Instituto de Investigación e Ingeniería Ambiental de la Universidad Nacional de San Martín, Buenos Aires, Argentina*

Figure 1. Geographic location of insect field populations used for assays: Salvador Mazza (22° 5′ S, 63° 7′ W) and Santa Rosa (23° 54′ S, 63° 11′ W), Salta, Argentina.

since 1975 and never exposed to insecticide treatment, was used as a reference for deltamethrin susceptibility.[11]

Captured insects and their progeny were raised at the laboratory under controlled temperature (28 ± 1 °C), humidity (50–70%) and photoperiod (12 : 12 h light : dark). A pigeon was weekly provided as a blood meal source.[12]

2.2 Chemicals

Technical-grade deltamethrin (99%) used for bioassay was obtained from Ehrestorfer, Germany. The analytical-grade acetone used for dilutions was purchased from JT Baker, Mexico.

2.3 Deltamethrin susceptibility determination

Lethal doses required to cause 50% mortality (LD_{50}) were determined according to the WHO protocol.[12] Bioassays consisted of the topical application of 0.2 µL of deltamethrin in acetone solution, and were replicated 3 times. The insecticide application was conducted by means of a 10 µL Hamilton syringe with automatic dispenser, on the dorsal abdomen of first-instar nymphs (1.2±0.2 mg). These were 3–5 days old and had been starved since eclosion. At least four doses in a range that produced between 10 and 100% mortality were applied, and control groups received only pure acetone. Insects were held at the same laboratory conditions for 24 h, when mortality was evaluated. To this end, treated insects were placed at the centre of a circular filter paper (11 cm diameter) and their behaviour was observed. Only those nymphs that were able to walk to the paper border, with or without mechanical stimulation, were considered alive.

2.4 Inheritance of deltamethrin resistance assays

Newly moulted adults were sexed and kept in individual flasks to ensure virginity until the assay began. Reciprocal mass crosses between the resistant (R) and susceptible (S) colonies were carried out to produce the F_1 generation. Three virgin couples were used for each cross, which led to four groups of testing: S × S, R × R, R♂ × S♀, R♀ × S♂. Newly born nymphs were used for toxicological studies, as in Section 2.3.

2.5 Data analysis

LD_{50}, resistance ratio (RR) and parallelism tests were conducted according to Robertson *et al.*,[13] using POLO plus software. The susceptible field strain was considered to represent the baseline for insecticide susceptibility for non-treated insects.

The degree of dominance, *D*, for resistance was calculated by the formula[14,15]

$$D = \frac{2\theta_3 - \theta_2 - \theta_1}{\theta_2 - \theta_1} \quad (1)$$

where $\theta_1 = \log(LD_{50})$ of the susceptible strain S, $\theta_2 = \log(LD_{50})$ of the resistant strain R and $\theta_3 = \log(LD_{50})$ of the heterozygous strains R × S. The *D* values range between −1 (complete recessivity) and 1 (complete dominance).

Effective dominance on survival was calculated as follows:[16]

$$D_{ML} = \frac{ML_3 - ML_1}{ML_2 - ML_1} \quad (2)$$

where ML_1, ML_2 and ML_3 represent the mortality percentages for the susceptible, the resistant and the R × S strains respectively. D_{ML} expresses the effective dominance at a given dose of use, and ranges between 0 (survival is recessive) and 1 (survival is dominant). The discriminant dose (DD) of deltamethrin was chosen as a reference for effective dominance in these colonies. This dose causes 100% mortality when used on the CIPEIN strain, and was established as DD = 2 ng insect^{-1}.

3 RESULTS

3.1 Deltamethrin baseline susceptibility

The susceptibility of the field reference population was evaluated and compared with that of the laboratory reference strain CIPEIN (Table 1). The overlapping of 95% confidence limits indicated that no significant differences were found between these colonies. In the absence of significant differences of deltamethrin toxicity between the laboratory and field susceptible insects, further calculations are expressed relative to the latter.

3.2 Inheritance of deltamethrin resistance

Probit analysis was suitable for bioassay results for every cross, as indicated by the non-significant values of chi-square analysis shown in Table 2. The R colony was highly resistant to deltamethrin, presenting a resistance ratio higher than 850-fold relative to the susceptible colony. Crosses between the susceptible and the resistant colonies presented virtually identical LD_{50} and resistance ratios, indicating no sex linkage on resistance. The F_1 generations were 25- and 35-fold more resistant than the susceptible colony (R♂ × S♀ and R♀ × S♂ respectively) (Table 2). A ratio between the LD_{50} of the resistant colony and the crosses' progeny showed that the latter are, respectively, 34- and 25-fold less tolerant to deltamethrin than the resistant parent colony. These results suggested that resistance is inherited as a semi-dominant trait.

Dose–response slopes were significantly different between the susceptible and the resistant colonies (Table 2), which implies that the response to the insecticide is different between these. While the S colony presents a quick response to dose increase, the R colony shows a smaller slope, which would explain the need to apply higher doses to obtain a measurable response to the insecticide application. The slopes of the R♂ × S♀ and R♀ × S♂ colonies were equal to that of the susceptible strain ($\chi^2 = 0.95$, df = 2), indicating the existence of no maternal effect on insecticide response.

Given that in this study the lethal doses did not differ between the R × S strains, data were pooled for degree of dominance

Table 1. Toxicity of topically applied deltamethrin to field (S) and laboratory (CIPEIN) susceptible colonies of *Triatoma infestans*

Colony	n[a]	LD_{50} (95% CL) (ng insect^{-1})[b]	RR (95% CL)[b]	Slope (± SE)	df[c]	χ^2[d]
CIPEIN	145	0.13 (0.11–0.15)	1	3.1 (±0.3)	2	0.96
S	145	0.07 (0.02–0.16)	0.55 (0.27–1.13)	1.4 (±0.3)	12	17.59

[a] Number of nymphs used for bioassays.
[b] Lethal doses 50% (LD_{50}), resistance ratios (RR) and 95% confidence limits (CL) calculated following Robertson *et al.*[13]
[c] Degrees of freedom.
[d] Chi-square is not significant ($P > 0.05$).

Table 2. Toxicity of topically applied deltamethrin to susceptible (S), resistant (R) and reciprocally mated insects' (R♂ × S♀; R♀ × S♂) progenies of field populations of *Triatoma infestans*

Colony	n[a]	LD_{50} (95% CL) (ng insect^{-1})[b]	RR (95% CL)[b]	Slope (± SE)	df[c]	χ^2[d]
S	145	0.07 (0.02–0.16)	1	1.4 (±0.3)	12	17.59
R	173	60.87 (20.95–1040.61)	856.84 (265.30–2767.35)	0.6 (±0.2)	13	20.72
R♂ × S♀	175	1.77 (0.91–3.15)	25.01 (10.49–59.64)	1.1 (±0.2)	15	17.96
R♀ × S♂	120	2.46 (0.87–6.09)	34.66 (11.74–102.34)	1.3 (±0.3)	3	0.46

[a] Number of nymphs used for bioassays.
[b] Lethal doses 50% (LD_{50}), resistance ratios (RR) and 95% confidence limits (CL) calculated following Robertson *et al.*[13]
[c] Degrees of freedom.
[d] Chi-square is not significant ($P > 0.05$).

calculations. The degree of dominance calculated using Stone's formula was virtually equal to zero ($D = -0.025$), suggesting that the resistance trait would be additive. Effective dominance calculations conducted using the reference discriminant dose showed that, at that dose level, resistance is incompletely dominant ($D = 0.66$).

4 DISCUSSION

Experimental results showed that the response of SR and RS hybrids to deltamethrin was intermediate between the highly resistant and the susceptible parent colonies, indicating some degree of semi-dominance for resistance. Moreover, lack of significant differences between the LD_{50} and resistance ratios of the reciprocally mated insects indicated no sex linkage on this trait. These results, in addition to degree of dominance calculations, suggest that resistance to deltamethrin in this colony of *T. infestans* is controlled by semi-dominant, autosomally inherited factors.

Similar results have been found for pyrethroid insecticides in the past. Collins[17] and Hardstone *et al.*[18] reported that pyrethroid resistance in *Tribolium castaneum* (Herbst) and *Culex pipiens quinquefasciatus* (Say), respectively, was autosomal and controlled by incompletely dominant inherited factors. Similarly, Ru *et al.*[19] found pyrethroid resistance inheritance to be autosomal and incompletely recessive in *Helicoverpa armigera* (Hübner). Also, a codominant and autosomal mechanism of resistance inheritance was reported for a laboratory strain of tobacco budworm *H. virescens* resistant to cypermethrin and thiodicarb.[20] In particular, deltamethrin inheritance has been found to be autosomal and incompletely recessive in *Cydia pomonella* (L.),[21] and incompletely dominant in *P. xylostella*.[22]

Even though information about the number of genes involved in resistance to deltamethrin is lacking from this study, previous studies from the authors' laboratory suggest that more than one gene is affecting this insecticide action in *T. infestans* from the Salvador Mazza area. Picollo *et al.*[3] demonstrated that pretreatment with a monooxygenase inhibitor (piperonyl butoxide) produced only a partial reversion of resistance in the pyrethroid-resistant insects from this area. Later, Santo Orihuela *et al.*[4] studied the relative contributions of monooxygenase and esterase to pyrethroid resistance in *T. infestans* from different areas of Argentina. These authors found an increased P450 monooxygenase and specific esterase activity in three populations from the Salvador Mazza area. These results are consistent with Collins[23] and Ru *et al.*,[19] who showed that the synergist piperonyl butoxide could not completely suppress pyrethroid resistance in *T. castaneum* and *H. armigera*, suggesting that more than one resistance mechanism was present.

In addition to the mode of inheritance of resistance, differences in fitness of insecticide-resistant insects could have a profound influence on its rate of evolution. Reproductive disadvantages in insecticide-resistant insects have been demonstrated for several species,[24–27] although that is not the case for every resistant insect population.[28–31] Preliminary observations suggest that the resistant and the susceptible colonies used for the reciprocal crosses present similar fecundity under laboratory conditions. Further studies on this aspect of *T. infestans* biology will provide evidence on the possible reproductive consequences of resistance and their contribution to a stable development of this phenomenon.

Knowledge on inheritance of resistance in field populations of *T. infestans* is of significant importance in control strategies of this Chagas disease vector. Considerations related to insecticide campaigns could allow management of allele frequencies and resistance development, achieving a sustainable control of this species. Further research on the genetics of resistance and its possible fitness costs is necessary to achieve a complete understanding of this phenomenon and its implications in insect control.

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