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Inheritance and heritability of deltamethrin resistance under laboratory conditions of *Triatoma infestans* from Bolivia

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Abstract

Background: Over the last few decades, pyrethroid-resistant in *Triatoma infestans* populations have been reported, mainly on the border between Argentina and Bolivia. Understanding the genetic basis of inheritance mode and heritability of resistance to insecticides under laboratory conditions is crucial for vector management and monitoring of insecticide resistance. Currently, few studies have been performed to characterize the inheritance mode of resistance to pyrethroids in *T. infestans*; for this reason, the present study aims to characterize the inheritance and heritability of deltamethrin resistance in *T. infestans* populations from Bolivia with different toxicological profiles.

Methods: Experimental crosses were performed between a susceptible (S) colony and resistant (R) and reduced susceptibility (RS) colonies in both directions (♀ x ♂ and ♂ x ♀), and inheritance mode was determined based on degree of dominance (DO) and effective dominance (D_{ML}). In addition, realized heritability (h^2) was estimated based on a resistant colony, and select pressure was performed for two generations based on the diagnostic dose (10 ng. i. a. /nymph). The F1 progeny of the experimental crosses and the selection were tested by a standard insecticide resistance bioassay.

Results: The result for DO and D_{ML} (< 1) indicates that resistance is an incompletely dominant character, and inheritance is autosomal, not sex-linked. The LD_{50} for F1 of ♀S x ♂R and ♂S x ♀R was 0.74 and 3.97, respectively, which is indicative of dilution effect. In the resistant colony, after selection pressure, the value of h^2 was 0.37; thus, the LD_{50} value increased 2.25-fold (F2) and 26.83-fold (F3) compared with the parental colony.

Conclusion: The inheritance mode of resistance of *T. infestans* to deltamethrin, is autosomal and an incompletely dominant character; this is a previously known process, confirmed in the present study on *T. infestans* populations from Bolivia. The lethal doses (LD_{50}) increase from one generation to another rapidly after selection pressure with deltamethrin. This suggests that resistance is an additive and cumulative factor, mainly in highly structured populations with limited dispersal capacity, such as *T. infestans*. This phenomenon was demonstrated for the first time for *T. infestans* in the present study. These results are very important for vector control strategies in problematic areas where high resistance ratios of *T. infestans* have been reported.

Keywords: *Triatoma infestans*, Inheritance, Heritability, Insecticide resistance, Deltamethrin, Control

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Background

The prevention of Chagas disease is primarily based on vector control, using mainly pyrethroid insecticides [1]. Successful control of Triatoma infestans in Brazil, Chile, Uruguay, and drastic reduction of it in large parts of Paraguay and Argentina, are both clearly indicative of its susceptibility to pyrethroid insecticides in its largest area of distribution [2]. By contrast, resistance to pyrethroids in T. infestans has been detected since the year 2000 [3] in several areas of Argentina, Bolivia and Paraguay [4–11]. Failure in vector control is considered to be due to low insecticide efficacy when applied to peridomestic structures, unsustainability of vector control interventions, and high levels of insecticide resistance, reported mainly on the border between Argentina and Bolivia, in the biogeographic region of the Gran Chaco [6, 9, 10, 12].

Genetic factors and intensive insecticide application are responsible for the rapid selection of resistance in many insects and mites [13]. Several factors influence the development of insecticide resistance in insect vectors: volume and frequency of insecticide applications (selective pressures), operational failures, and inherent bionomical characteristics of the insect species involved, such as life cycle, abundance, rapid reproduction, and insect migration [14].

Laboratory models are key to understanding the underlying genetics of inheritance and heritability of insecticide resistance. Conclusions drawn from models are fundamental to improve vector management and resistance monitoring [15]. The inheritance mode of pyrethroid resistance was studied in T. infestans, and it showed that resistance is autosomal and shows incomplete dominance, involving at least three genes [16, 17]. Therefore, this study aims to characterize inheritance and heritability of insecticide resistance of T. infestans populations from Bolivia to deltamethrin with different toxicological profiles.

Methods

Insects

We used the F2 generation of three colonies of T. infestans from Bolivia, with different toxicological profiles to deltamethrin, previously characterized by Gomez et al. [18]: a susceptible colony (S) from San Silvestre (19°21'21"S/62°34'10"W) Santa Cruz Department; a reduced susceptibility colony (RS) from Rancho Nuevo (19°26'22"S/62°34'05"W) Santa Cruz Department, and a resistant colony (R) from Villa Montes (21°09'02"S/63°21'56"W) Tarija Department. Details of lethal dose 50 (LD_{50}) and resistance ratio 50 (RR_{50}) are shown in Table 1.

The calculation of the RR_{50} of the experimental cross was performed with the susceptible colony (S), because during the study carried by Gomez et al. [18], this colony was significantly more susceptible than the reference lineage (SRL) of T. infestans from Centro de Investigaciones de Plagas e Insecticidas (CIPEIN) (X^2 $p < 0.05$). However, the calculation of the RR_{50} for estimation of heritability was performed with the SRL of T. infestans.

Experimental matings

Resistance inheritance (cross)

To study inheritance, male and female fifth-instar nymphs were first identified and maintained individually, until the imaginal moult. Then, triatomine couples were formed, and reciprocal crosses were carried out as follows: ♀S x RS♂; S♂ x ♀RS; ♀S x R♂ and S♂ x ♀R. All couples were maintained in plastic cages (5 cm diameter, 10 cm height), feeding was carried weekly with chicken blood (Gallus gallus) (ethical approval of FIOCRUZ No 41/14-2). The F1 progeny was tested through a standard bioassay.

Estimation of realized heritability

The evaluation of heritability of resistance was performed with the F1 generation of the resistant colony from Villa Montes Tarija Department ($RR_{50} = 129.12$), and the individuals selected were those that survived the

Table 1 Toxicological profiles to deltamethrin for colonies of T. infestans: *susceptible (S), *reduced susceptibility (RS), *resistant (R) and reciprocally cross progenies: ♀S x ♂RS; ♂S x ♀RS; ♀S x ♂R; ♂S x ♀R

Population	N°	Slope +/− SD	X^2 (df)	P	LD_{50} (95 % CI)	RR_{50} (95 % CI)	DO	D_{ML}
*S	300	1.97+/− 0.05	0.51 (6)	0.00	0.26 (0.21–0.32)	-	-	-
*RS	390	4.43+/− 0.02	2.41 (10)	0.01	2.09 (1.93–2.27)	5.04		
*R	360	2.25 +/− 0.04	3.05 (9)	0.04	54.23 (45.54–63.32)	129.12		
♀S x ♂RS	210	5.44 +/− 0.76	5.34 (4)	0.23	0.65 (0.55–0.76)	2.64 (2.12–3.29)	−0.08	−1.37
♂S x ♀RS	300	4.33 +/− 0.43	4.30 (7)	0.04	1.30 (1.18–1.43)	5.27 (4.22–6.52)	0.54	−8.07
♀S x ♂R	360	4.44 +/− 0.46	3.18 (7)	0.21	0.74 (0.67–0.81)	2.99 (2.39–3.73)	−0.61	0.00
♂S x ♀R	210	5.86 +/− 0.78	3.17 (6)	0.40	3.97 (3.62–4.30)	16.06 (12.93–19.94)	0.02	0.76

*: Colony characterized by Gomez et al. [18]; N°: number of individuals used; SD: standard deviation; X^2: chi-square test; df: degrees of freedom; P: probability value; LD_{50}: insecticide dose that killed 50 % of the population [ng/insect]; CI: confidence intervals; RR: resistance ratio; ♀: female; ♂: male; DO: Degree of dominance; D_{ML}: effective dominance

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application of the diagnostic dose of deltamethrin (10 ng a.i./nymph) for successive generations (F2 and F3 survivors); they were then tested by standard bioassays.

Bioassays

Serial dilutions of technical grade deltamethrin were prepared in acetone. For each concentration, three repetitions were carried out with ten first-instar nymphs, descendant from each of the experimental crosses (the corresponding F generation for each experiment) (five days old, fasting, weight = 1.2 ± 0.2 mg). Diluted insecticide (0.2 μL) was applied on the dorsal abdomen, according to the procedures recommended by the World Health Organization - WHO [19] and Pessoa [20]. For each experimental cross, at least eight doses of insecticide were applied in order to kill between >0 % to <100 % of the individuals (0.35 to 1500.00 ng a. i./nymph). Acetone was applied to the control group. Mortality was assessed 72 h after application, and it was determined by the inability or lack of coordination of the nymphs to move from the center to the edge of the filter paper (7 cm in diameter). Signs of paralysis and lack of response to external stimuli were also taken into consideration. During and after the experiment, the insects were kept under controlled temperature and humidity conditions (25 °C ±1 °C; 60 % ±10 % RH).

Data analysis

Toxicological data

Data from dose response tests from each experiment were analyzed using the PoloPlus software, version 2.0 [21]. Estimations were made of the slope, the lethal doses required to kill 50 % of treated individuals (LD_{50}) and confidence intervals (CIs.). The resistance Ratio (RR_{50}) was calculated by dividing the LD_{50} value of each experimental cross by the LD_{50} value of the susceptible colony. Parallelism tests were also conducted according to Robertson et al. [22].

Degree of dominance

Degree of dominance (DO) for resistance was calculated according to Stone [23] and Preisler et al. [24] using the following formula:

$$DO = \frac{2X_3 - X_2 - X_1}{X_2 - X_1}$$

Where: X_1 = log (LD_{50}) of the susceptible colony (S); X_2 = log (LD_{50}) of the RS or R colony and X_3 = log (LD_{50}) in the reciprocal progeny (F1).

The level of dominance ranges from 0 to 1.0 (i.e. values below 1.0 indicate complete recessiveness and values equal to 1.0 indicate complete dominance).

Effective dominance

Effective dominance (D_{ML}) was estimated according to Bourguet et al. [25] and Abbas et al. [26] using the following formula:

$$D_{ML} = \frac{MX_3 - MX_1}{MX_2 - MX_1}$$

Where: MX_1, MX_2 and MX_3 represent the mortality percentages for the susceptible, the RS or the R colony and the reciprocal cross progeny (F1), for doses that cause 100 % mortality of the SRL (2.76 ng a. i./nymph treated). D_{ML} expresses effective dominance at a given dose of use, and ranges between 0.0 (survival is recessive) and 1.0 (survival is dominant).

Estimation of realized heritability

Following the method of Falconer et al. [27] and Tabashnik [28], realized heritability (h2) of deltamethrin resistance was estimated as follows:

$$h^2 = \frac{R}{S}$$

In the above equation, R (selection response) was estimated as follows:

$$R = \frac{Log_{final}\,LD_{50} - Log_{initial}\,LD_{50}}{N}$$

Final LD_{50} is the LD_{50} of the population after 2 selected generations; initial LD_{50} is the LD_{50} of the field population before selection and N is number of generations selected with deltamethrin.

Whereas, S (selection differential) was calculated as follows:

$$S = i x \sigma p$$

Where i means intensity of selection calculated according to Falconer et al. [27],

σp means phenotypic standard deviation calculated as follows:

$$\sigma p = [(initial\,Slope + final\,Slope)\,0.5]^{-1}$$

Results

Inheritance of resistance (cross)

The LD_{50} values to deltamethrin of all reciprocal crosses carried out of *T. infestans*, (♀S x ♂RS; ♂S x ♀RS; ♀S x ♂R and ♂S x ♀R) were significantly different and inferior than their parental RS and R colonies (p < 0.05). This is indicative that deltamethrin resistance in *T. infestans* is inherited autosomal, not sex-linked (Table 1).

The LD_{50} progeny values of ♀S x ♂R and ♂S x ♀R were 0.74 and 3.97, respectively (Table 1), showing that they are 46.11-fold and 8.34-fold less tolerant to deltamethrin than

their parental insects of the resistant colony. These results indicate a dilution effect among the resistant and susceptible colonies.

Degree of dominance (DO) values for reciprocal crosses were < 1. This result indicated that the deltamethrin resistance character in *T. infestans* is incompletely dominant. The results of effective dominance (D_{ML}) (<1) suggested that resistance is a recessive character at the discriminant doses used for deltamethrin (Table 1).

Estimation of realized heritability

After 2 generations of continuous selection of *T. infestans* with deltamethrin, it was observed that the LD_{50} value increased from 54.23 to 121.93 (F2) and 1455.32 (F3); it is 2.25-fold and 26.83-fold more resistant compared with the parental resistant colony (Table 2). The value calculated for estimation of realized heritability (h^2) was 0.37 (Table 3). These results indicate increased resistance to deltamethrin from one generation to another, under pressure selection, under laboratory conditions.

Discussion

For *T. infestans*, few studies have been conducted about resistance inheritance. Cardozo et al. [16] and Germano et al. [17], suggested that deltamethrin resistance of *T. infestans* is autosomal, incompletely dominant, and the maternal effects are null. This fact was confirmed during our study; regardless of the direction where crossing was performed (female or male; susceptible colony with resistant or with reduced susceptibility colony), degree of dominance and effective dominance values were < 1, even lower to 0, indicating incomplete dominance. The LD_{50} values to deltamethrin for reciprocal crosses were significantly different and lower than their parental colonies ($p < 0.05$).

Khan et al. [29] stressed that intense selection pressure leads to an increase in resistant genotypes in the population due to the removal of susceptible individuals, increasing the frequency of resistant individuals in a population. However, epistatic interactions can occur, mainly in populations with inbreeding, when each allele contributes to resistance, and the introduction of a susceptible allele dilutes the effect under laboratory conditions [30]. Thus, during our study, the LD_{50} values of

experimental crosses among resistant and susceptible colonies were 0.73 (R♀ x S♂) and 3.98 (S♀ x R♂) (Table 1), showing a 46.11-fold and an 8.34-fold decrease in the LD_{50} values when compared with resistant parental insects (LD_{50} = 50.23). This suggests that there is a dilution effect of the resistant character to deltamethrin under laboratory conditions.

Several studies demonstrated high levels of structuring of *T. infestans* populations [31–35, 36] and indicated limited capacity of active dispersal and restricted interbreeding. Germano et al. [37] suggested that geographical structure is present at the microgeographic level, and demonstrated that *T. infestans* populations in different dwellings in the same area have different toxicological profiles to deltamethrin. Selection pressure for insecticide application has a greater effect in populations with limited capacity of dispersal, as is the case of *T. infestans*, than on populations with high dispersal ability. The persistent bug populations that survived insecticide application at local spatial scales support two distinct, but equally worrying scenarios. In the first scenario, operational failures may hinder the control of Triatomine populations, as a result of low quality of the spraying technique and/or low efficacy of pyrethroids, especially in peridomestic structures. In the second scenario, difficulty in control may be due to the intrinsic features of Triatomine bugs, which make them resistant to chemical agents. [32, 38–40].

Different toxicological profiles and mechanisms of resistance were reported for *T. infestans* [7, 8, 11, 41]. Mutation of two different alleles of knockdown resistance (*kdr*) were reported from different populations in the Gran Chaco region in Argentina [7, 41], spanning a different geographical distribution. Coincidently, the resistant population used for the experiment of heritability was collected in the Gran Chaco region, where it can be seen that the LD_{50} value increased from 54.23 (parental) to 121.93 (F2) and 1455.32 (F3) (Table 2). These results suggest that resistance to deltamethrin in *T. infestans* could increase rapidly. Despite their long life cycle, only two generations of continuous laboratory selection were required to increase the level of resistance 26.83-fold. The rapid selection and high levels of resistance in this population are likely to be caused by *kdr* mutation.

Table 2 Toxicological profiles to deltamethrin in survivors of *T. infestans* resistant colony (F1 and F2), after application of deltamethrin (10 ng a.i./nymph)

Population cross	N°	Slope +/− SD	X^2(df)	P	LD_{50} (95 % CI)	RR_{50} (95 % CI)
CIPEIN (SRL)*	240	2.83+/−0.04	3.43 (4)	0.51	0.42 (0.35–0.49)	-
Resistant (Parental) Colony*	360	2.25 +/− 0.04	3.05 (9)	0.04	54.23 (45.54–63.32)	129.12 (104.32–166.38)
Surviving F1	210	1.68 +/− 0.42	0.59 (5)	0.01	121.93 (93.65–161.66)	299.23 (222.48–402.47)
Surviving F2	180	1.60 +/− 0.45	0.56 (4)	0.00	1455.32 (1022.71–3914.21)	3571.45 (2099.09–6076.53)

SRL: susceptible reference lineage*: Strain characterized by Gomez et al. [18]; N°: number of individuals used; SD: standard deviation; X^2: chi-square test; *df*: degrees of freedom; P: probability value; LD_{50}: insecticide dose that killed 50 % of the population [ng/nymph]; CI: confidence intervals; RR: resistance ratio

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Table 3 Estimate of heritability (h^2) to deltamethrin resistance in survivors of *T. infestans* after insecticide application under laboratory conditions

N° of generations selected	Estimation of mean selection response per generation			Estimation of mean selection differential per generation					
	Initial log LD_{50}	Final log LD_{50}	Response to selection (R)	i	Initial slope	Final slope	σp	Selection differential (S)	h^2
2	1.73	3.16	0.72	3.79	2.25	1.60	0.52	1.97	0.37

i = intensity of selection [44]; σp = phenotypic variation; h^2 = Estimation of heritability

Estimation of heritability (h^2) is a proportion of phenotypic variation accounted by additive genetic variation, which may decrease either due to a decrease in additive genetic variance or to an increase in environmental variance [27]. During our study, the value for estimation of heritability (h^2) to deltamethrin was 0.37 (Table 3). This lower value was most likely caused by lower phenotypic variation, mainly environmental variation, which indicates the presence of resistant alleles in the study colony [42]. Similarly, the selection was done under laboratory conditions; it does not completely reflect the development of resistance in the field population because of homogeneous environmental conditions [28]. In addition, polygenic and monogenic resistance may occur naturally in insect populations [29]. However, monogenic resistance is less likely to occur under laboratory selection, given the absence of rare variants that may be present in natural insect populations [43, 44].

The laboratory experiments and the use of only one insecticide (deltamethrin) impose limitations compared to field conditions. However, most studies evaluating the susceptibility of insects in the laboratory used deltamethrin (technical grade) as a standard insecticide, because this molecule is stable and there is a great amount of knowledge available about it. As the results in this study only used deltamethrin, it could be reasonably extrapolated to other pyrethroids, but not to other active ingredients (i.e. carbamates).

Moreover, the estimated heritability values provide evidence for the potential of resistance development in the future [15, 29, 30, 43]. Thus, the results of estimation of realized heritability allow the prediction of the number of generations required for the population to increase the level of resistance [28]. It should be noted that this is the first study on estimation of realized heritability in *T. infestans* to deltamethrin, and despite the long life cycle (around 6 months for adults), the values of LD_{50} could be increased considerably (26.83-fold) within two generations. Characterizing the mode of inheritance and determining the estimation of the h^2 value is an essential tool for assessing sustainability of insecticides on the pest population and vectors of public health importance for resistance management [45]. Therefore, more long-term studies should be conducted to clarify the selection process in bugs, because it is very important for control vector management.

Conclusion

The results obtained in our study indicate that (1) the inheritance mode of deltamethrin resistance of *Triatoma infestans*, under laboratory conditions, is autosomal and an incompletely dominant character. This is a previously known process, and it was confirmed in other *T. infestans* populations from Bolivia in this study; (2) the lethal doses (LD_{50}) and resistance ratio increase from one generation to another rapidly, after selection pressure with deltamethrin. This suggests that resistance is an additive and cumulative factor, mainly in highly structured populations with limited dispersal capacity, such as *T. infestans*. This phenomenon was demonstrated for the first time for *T. infestans* under laboratory conditions in the present study. These results are very important for vector control programs in problematic areas where high resistance ratios of *T. infestans* have been reported.

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