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Kdr mutations in *Triatoma infestans* from the Gran Chaco are distributed in two differentiated foci: Implications for pyrethroid resistance management

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ABSTRACT

Point mutations in the voltage-gated sodium channel, the primary target of pyrethroid insecticides, have been associated with the resistance in *Triatoma infestans*, an important vector of Chagas' disease. Hence, the sustainability of vector control programs requires the implementation of resistance management strategies.

We determined the sensitivity of the molecular assays previously designed for early resistance detection to be used in pooled samples from a wide area of the endemic region, and validated them for their routine use in control campaigns for the monitoring of insecticide resistance in *T. infestans*. Consequently, we used these methods to examine the distribution of resistance-associated mutations in the sodium channel gene in populations of *T. infestans* from the Argentinean and Bolivian Gran Chaco.

The PASA and REA assays tested proved sensitive enough to detect *kdr* SNPs in pooled samples, indicating these assays are suitable for routine screening in insecticide resistance surveillance. Two geographically differentiated foci were detected in *T. infestans* populations from the Argentinean and Bolivian Gran Chaco, with populations on the Bolivian-Argentinean border carrying L1014F mutation, and those from the Argentinean Chaco carrying L925I mutation. In all highly resistant populations analyzed, one of both *kdr* mutations was present, and toxicological assays determined that all pyrethroid resistant populations analyzed herein were sensitive to fenitrothion.

The principal cause of pyrethroid resistance in *T. infestans* from the Gran Chaco ecoregion is *kdr* mutations in the sodium channel. Different levels of resistance occur in different populations carrying identical mutation, suggesting the existence of contributory mechanisms.

1. Background

Eight million people in Latin America are infected with Chagas' disease, and a fifth of the population of the region remains at risk. Thirty–forty percent of those infected develop cardiomyopathy and/or digestive syndromes (Rassi et al., 2010). The causative agent of the disease, the protozoan *Trypanosoma cruzi*, is primarily transmitted to humans through the feces of triatomine bugs, and enters broken skin as their itchy bites are scratched. The most important Chagas' vector in South America is *Triatoma infestans*. Given the absence of vaccines and efficient treatments in the chronic stage, reduction of triatomine population is the method of choice for the control of disease transmission. In 1991, the Southern Cone Initiative (SCI), established by the authorities of the Southern Cone

Abbreviations: *kdr*, knockdown resistance; PASA, PCR amplification of specific alleles; REA, restriction endonuclease assay; SCI, southern cone initiative; SNP, single nucleotide polymorphism; TiNaV, *T. infestans* sodium channel; M, madrejones; LE, la esperanza; LG, la gerónima; EJ, el juramento; EM, el malá; PG, Pampa Grande; TN, Tierras Nuevas; VC, Villa El Carmen; Y, Yacuiba.

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Nations, successfully reduced Chagas' transmission by the elimination of triatomines from dwellings, and enforced Chagas' screening of blood donors (Dias, 2007; Gurtler, 2009).

Gran Chaco ecoregion covers 1.1 million km^2 distributed in Argentina, Paraguay and Bolivia (Cabrera, 1971). In this large semiarid region, the vectorial transmission of Chagas' disease has not yet been stopped, even though the vector control strategies with the spraying of pyrethroid insecticides were similar to those used in other areas (Gurtler, 2009; Gurtler et al., 2007). SCI recognized as a priority the determination of the causes for the failures in the elimination of *T. infestans* from the Gran Chaco (Dias, 2007). Even though it would be a multicausal problem, including socioeconomic factors, resistance to pyrethroid insecticides in populations of *T. infestans* from the Gran Chaco contributes to the failures in spraying with pyrethroids (Picollo et al., 2005).

An initial focus of high pyrethroid resistance in *T. infestans* was identified on the Argentinean-Bolivian border (Picollo et al., 2005; Santo-Orihuela et al., 2008) and more recent findings documented another focus of high resistance in the Argentinean Chaco Province, at the center of the Gran Chaco (Carvajal et al., 2012). Toxicological, biochemical and genetic evidence accumulated to date points to multiple origins of the resistance, rather than the spread of a single point resistant population (Capriotti et al., 2014; Fabro et al., 2012; Germano et al., 2012).

Pyrethroids are neurotoxins that target the voltage-dependent sodium channel (Na_v), a membrane protein involved in the transmission of the action potential. Single nucleotide polymorphisms (SNPs) in the Na_v gene generate amino acid changes in the protein, which reduce the sensitivity of the molecule to pyrethroids. These resistance-associated mutations are called knockdown resistance (*kdr*) alleles (Dong et al., 2014). Although several mechanisms of insecticide resistance have been described in *T. infestans* and other insects (Santo-Orihuela et al., 2008; Capriotti et al., 2014; Fabro et al., 2012; Pedrini et al., 2009), sodium channel mutations occur whenever the highest levels of resistance are observed (Dong et al., 2014). Our group recently verified the existence of two different *kdr* SNPs in *T. infestans* populations in Argentina, L1014F in a population near the Bolivian border (Salta Province) (Fabro et al., 2012), and L925I in a population from the center of the Argentinean Chaco Province (Capriotti et al., 2014). Furthermore, molecular assays to test the presence of *kdr* mutations in the field were developed (Capriotti et al., 2014; Fabro et al., 2012). As was observed in a range of insect species, an intron in *TiNa$_v$* gene is located very close to the L1014F mutation site (Fabro et al., 2012). We have also observed that this region of the gene shows a degree of variation in non-coding positions (silent mutations) between different individuals and strains (Capriotti et al., 2014). These facts would affect the performance of any assay that uses primer binding sites within the region, as is the case of the methods developed recently. Hence, a validation for its use in different population is required.

The main objectives of the present work are to analyze the presence and distribution of the two *kdr* SNPs in resistant populations from the Gran Chaco; if both mutations co-exist in the same area, or if they are distributed in differentiated foci. Furthermore, we validated molecular assays for their use in resistant management strategies, with pooled samples from a range of populations of *T. infestans*.

2. Methods

2.1. Ethics statement

Pigeons were housed, cared for, fed and handled in accordance with resolution 1047/2005 (CONICET) regarding the national reference ethical framework for biomedical research with laboratory, farm, and nature collected animals. This is in agreement with the standard procedures of the Office for Laboratory Animal Welfare, Department of Health and Human Services, NIH, and the Directive of the European Parliament (2010/63/EU), in relation to the use of animals for scientific research. Biosecurity considerations are in accordance with CONICET (Res 1619/2008) and WHO Biosecurity Handbook (ISBN 92 4 354 6503). According to those rules, approval from the CONICET Ethic Committee was not required for this work. The collection of insects in dwellings was performed in agreement with the Argentinean National Health Ministry's ethical requirements. The inhabitants of each domicile were informed about the objectives, and the protocol of the procedures was used. Collections were not performed unless they expressed their consent. Vector Control Program officers familiarized with the population participated as mediators and collaborated with insect sampling.

Fig. 1. Map of Northern Argentina and Southern Bolivia showing the small villages where *T. infestans* were collected. A: Bolivian–Argentinean border: LE: La Esperanza; LG: La Gerónima; EM: El Malá; EJ: El Juramento; PG: Pampa Grande. B: Chaco Province. M: Madrejones; VC: Villa El Carmen; TN: Tierras Nuevas; Y: Yacuiba. Scale Bar: 100 km.

2.2. Insect populations and strains

A susceptible strain (CIPEIN strain) raised since 1975 with no exposure to insecticides was used as a reference, as its response to deltamethrin had been repeatedly verified with susceptible field strains (Toloza et al., 2008; Germano et al., 2010; Castro et al., 1976). The field *T. infestans* specimens were collected in the Argentinean localities of Madrejones (M) (22°02′ S, 63°37′ W) (Salta Province), La Esperanza (LE) (26°03′ S, 60°27′ W) (Chaco Province), La Gerónima (LG) (26°04′ S, 60°16′ W) (Chaco Province), El Juramento (EJ) (25°54′ S, 60°24′ W) (Chaco Province), El Malá (EM) (25°56′ S, 60°27′ W) (Chaco Province), Pampa Grande (PG) (27°06′ S, 60°59′ W) (Chaco Province), and the Bolivian localities of Tierras Nuevas (TN) (21°44′ S, 63°33′ W) (Santa Cruz Department), Villa El Carmen (VC) (21°47′S, 63°34′ W) (Santa Cruz Department), and Yacuiba (Y) (22°01′ S, 63°40′ W) (Tarija Department) (Fig. 1). Insects were collected inside dwellings, and further generations of the field-collected insects were raised as previously described (Fabro et al., 2012). Table 2 specifies the year of collection from each population and the number of generations raised in the laboratory for the individuals used in this work.