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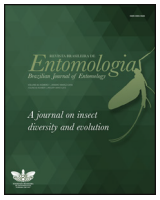

Genetic diversity and *Kdr* mutations of natural *Aedes* (*Stegomyia*) *aegypti* (Diptera: Culicidae) populations of Brazil

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ABSTRACT

Aedes (*Stegomyia*) *aegypti* is an important vector of dengue, yellow fever, chikungunya and Zika virus. It is well known that resistance monitoring and genetic diversity data help designing the vector control programs. This study aimed to evaluate resistance to pyrethroids (PYs) through the frequency of *kdr* mutations Val1016Ile and F1534C, and the genetic variation of the mitochondrial gene ND4 in six natural populations of *A. aegypti* from Paraná – Brazil. Adults were obtained from eggs collected from Alvorada do Sul, Marilena, Maringá, Nova Londrina, Paranavaí and São Carlos do Ivaí. From these adults, 345 were used to identify the 1016 and 1534 sites, and 120 were used to perform the ND4 gene analysis. The studied populations from Paraná showed PYs resistance, low gene flow and genetic diversity. Additionally, a relationship was observed among the haplotypes of populations from the Amazon and Southeastern Brazil, Peru, Mexico, and North America.

Introduction

Aedes (*Stegomyia*) *aegypti* (Linnaeus 1762) is an African Culicidae (Christophers, 1960) widely distributed in tropical and subtropical regions (Kraemer et al., 2015, 2019). In Brazil, it is the main vector of dengue, chikungunya, Zika virus, and it participates in the urban transmission of yellow fever (Lowe et al., 2018; Tanabe et al., 2018; Silva et al., 2020; Castro et al., 2021). *A. aegypti* successfully settles in urban areas where it aggravates the transmission of known and emergent arboviruses of public health concern (Forattini, 2002; Hotez and Murray, 2017; Girard et al., 2020). Since there is a lack of effective vaccines or antiviral drugs against the arboviruses that *A. aegypti* transfers, suppressing mosquito populations is the main mechanism for reducing or preventing virus transmission (Achee et al., 2015). Control can be done using chemical and non–chemical-based tools, such as elimination of potential breeding sites and insecticide applications (Garcia et al., 2018). The application of chemical insecticides is still a practice implemented for the management of *A. aegypti* populations, essentially in epidemic situations, although it is not the most effective strategy (Fernandes Bellinato et al., 2016). Pyrethroids – PYs are active ingredients of indoor spraying formulations and used to be compounds included in governmental control programs due to their knock-down effect and low toxicity to mammals (WHO, 2005; Braga and Valle, 2007). Because of the intensive use of PYs, *A. aegypti* populations of several countries have developed resistance to these compounds (Hernandez et al., 2021; Saavedra-Rodriguez et al., 2021). Even though the large-scale use of PYs has been restricted, resistance in mosquitoes is still being identified (Macoris et al., 2018). Similarly, resistance to pyrethroids has been reported in other arthropods of public health concern like *Triatoma infestans* (Klug) (Gaspe et al., 2021; Dulbecco et al., 2022), *Lutzomyia longipalpis* (Lutz & Neiva) (Alexander et al., 2009), *Rhipicephalus microplus* (Cossío-Bayúgar et al., 2018; Kumar et al., 2020), and *Pediculus humanus capitis* (Larkin et al., 2020). In Brazil, resistance to PYs has been reported since the 1990s in mosquitoes and, currently, it continues to be recorded in different areas of the country (da-Cunha et al., 2005; Linss et al., 2014; Valle et al., 2019; Itokawa et al., 2021).

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PYs prompt a disruption of the action potentials along the axon by the prolongated opening of the channels (Bloomquist, 1996; Chen et al., 2020) in the voltage–gated sodium channel (vgsc) of the central and peripheral nervous system, which is a transmembrane protein, made up of four homologous domains (I - IV), each containing six segments (S1 - S6) (Catterall, 2014). The PYs effect is called knockdown and is characterized by the paralysis of the insect (Davies et al., 2007). The knockdown resistance (*kdr*) is caused by conformational changes throughout the vgsc and substitutions of a single amino acid (García et al., 2009; Kawada et al., 2009). In *A. aegypti* the amino acid variation has been recorded at positions G923V, L982W, I1011M, V1016G (Brengues et al., 2003), I1011V (Saavedra-Rodriguez et al., 2007), D1763Y (Chang et al., 2009), S989P (Srisawat et al., 2010), T1520I (Kushwah et al., 2015), V410L (Haddi et al., 2017), F1534C (Yanola et al., 2011), and V1016I (Saavedra-Rodriguez et al., 2007). The V1016I mutation involves the substitution of a valine for isoleucine at position 1016 in the IIS6 region, and the F1534C mutation encompasses the replacement of a phenylalanine for a cysteine at position 1534 in the IIIS6 region of vgsc (Linss et al., 2014). Simultaneous occurrence of *kdr* mutations V1016I and F1534C also happens as registered in different populations of *A. aegypti* from Brazil (Linss et al., 2014; Macoris et al., 2018), Cayman Islands (Harris et al., 2010), Cuba (Bariami et al., 2012), Venezuela (Alvarez et al., 2015), Puerto Rico (Ponce-García et al., 2016), Argentina (Barrera-Illanes et al., 2023), Jamaica (Francis et al., 2017) and Mexico (Saavedra-Rodriguez et al., 2018). In addition to the flow of PY-resistant mosquitoes, migration of new species of medical importance may occurs between neighboring regions and countries (Barrera-Illanes et al., 2023; Mills, 2020).

The mitochondrial gene ND4, which codifies the subunit 4 of the NADH dehydrogenase enzyme, along with the cytochrome c oxidase subunit 1 – COI, and internal transcribed spacer - ITS2, have been considered efficient molecular markers to study the genetic variability of natural populations of *Aedes* populations (Al Ali et al., 2016; Khater et al., 2021; Escobar et al., 2022). ND4 has been used in multiple populations from Brazil (Bracco et al., 2007; Paduan and Ribolla, 2008; Lima Júnior and Scarpassa, 2009; Bona et al., 2012; Twerdochlib et al., 2012), Mexico (Gorrochotegui-Escalante et al., 2002), Peru (Costa-Da-Silva et al., 2005; Yáñez et al., 2013), Venezuela (Herrera et al., 2008; Urdaneta-Marquez et al., 2008), Bolivia (Paupy et al., 2012), Colombia (Caldera et al., 2013; Aguirre-Obando et al., 2015), and Chile (Núñez et al., 2016). Therefore, this study aimed to recognize the PYs resistance considering the frequency of *kdr* V1016I and F1534C mutations, as well as to understand the genetic variation of the natural populations of *A. aegypti* from Paraná – BR according to the analysis of ND4 mitochondrial gene that helps improve decisions towards the implementation of vector management and control strategies.

Methods

Sampling

Aedes spp. eggs were collected in 2013 and 2014 by staff members of the Dengue Vector Control Program using the ovitrap proposed by Fay and Eliason (1966) in six municipalities of the State of Paraná in Brazil: Alvorada do Sul, Marilena, Maringá, Nova Londrina, Paranavaí, and São Carlos do Ivaí. Ovitraps were analyzed in the Laboratory of Morphology and Physiology of Culicidae and Chironomidae - LAMFIC[2] in the Department of Zoology at the Federal University of Paraná - UFPR.

Taking into account that this ovitrap model is suitable for collecting both *Aedes albopictus* and *A. aegypti* eggs, it was necessary to separate the species after obtaining the adults. Eggs were reared under controlled temperature (25 ± 1 ºC), humidity (80 ± 10%) and photoperiod (12h), according to standard laboratory protocol. Populations from Maringá and Alvorada do Sul municipalities were clustered into six regions each, according to their field collection (Table 1). Adults were sorted individually in ethanol 99% after identification according to the key of Forattini (2002) and kept at -20°C until molecular analysis. For this study, males of *A. aegypti* of the F_0 generation were used.

V1016I and F1534C mutations were analyzed from 345 males of the six municipalities. From these samples, 120 males were randomly selected to identify the ND4 gene. DNA extraction for all analyses was performed individually using the protocol described in Bona (2012). The identification of these mutations was carried out in adults due to the greater use of PYs to control the vector during the adult stage. This does not disregard resistance to PYs during immature stages of the vector (Koou et al., 2014; Saha et al., 2019).

Table 1
Genotype frequencies in 1016 and 1534 sites of the VSC locus, and HWE test of *Aedes aegypti* from six municipalities of state of Paraná - Brazil.

Municipality (Year of field collection)	Region	Code	N		Genotype frequencies						HWE test
			ND4	*kdr*	SS	SR1	SR2	R1R1	R1R2	R2R2	$X^{2(df)*}$
Alvorada do Sul - AS (2014)		**AS**	**13**	**114**	**0.018**	**0.026**	**0.307**	**0.053**	**0.272**	**0.325**	**9.2**
	North Zone	ZN	-	20	0.000	0.000	0.300	0.300	0.389	0.400	
	Southern Zone	ZS	-	18	0.000	0.000	0.278	0.000	0.000	0.333	
	Eastern Zone	ZL	-	19	0.000	0.100	0.500	0.000	0.000	0.400	
	Western Zone	ZO	-	18	0.000	0.000	0.175	0.000	0.000	0.825	
	Central Zone	CT	-	20	0.000	0.000	0.150	0.000	0.600	0.250	
	Povoado Esperança	PE	-	19	0.263	0.263	0.211	0.000	0.053	0.211	
Maringá - MG (2013)		**MG**	**18**	**110**	**0.027**	**0.100**	**0.273**	**0.009**	**0.173**	**0.418**	**4.2**
	Regional 1	R1	-	20	0.000	0.150	0.300	0.060	0.150	0.350	
	Regional 2	R2	-	18	0.000	0.111	0.167	0.000	0.000	0.722	
	Regional 3	R3	-	20	0.000	0.150	0.450	0.000	0.300	0.100	
	Regional 4	R4	-	12	0.000	0.083	0.583	0.000	0.083	0.250	
	Regional 5	R5	-	20	0.050	0.100	0.100	0.000	0.050	0.700	
	Regional 6	R6	-	20	0.000	0.000	0.150	0.000	0.400	0.350	
Marilena - MR (2014)	-	**MR**	**13**	**33**	**0.061**	**0.091**	**0.394**	**0.030**	**0.182**	**0.242**	**1.1**
Nova Londrina - NL (2014)	-	**NL**	**19**	**30**	**0.100**	**0.133**	**0.333**	**0.067**	**0.267**	**0.100**	**2.0**
Paranavaí - PV (2013)	-	**PV**	**28**	**28**	**0.000**	**0.000**	**0.179**	**0.107**	**0.321**	**0.393**	**3.1**
São Carlos do Ivaí – SC (2013)	-	**SC**	**29**	**30**	**0.000**	**0.200**	**0.167**	**0.161**	**0.133**	**0.333**	**8.0**

*$(p < 0.05)$

Genotyping sites 1016 and 1534

Both sites, 1016 and 1534, were individually genotyped by allele-specific Polymerase chain reactions (AS-PCR) following the protocols described by Linss et al. (2014). Primers used to determine the presence of V1016I mutation were: wild allelo 1016Val: 5'-GCG GGC AGG GCG GCG GCG GGG GGG CCA CAA ATT GTT TCC CAC CCG CAC CGG -3', the mutant allele 1016I: 5'-GCG GGC ACA AAT TGT TGT TTC CCA CCC GCA CTG A -3', and a common one for both alleles: 5'-GGA TGA ACC GAA ATT GGA CAA AAG C -3" (Saavedra-Rodriguez et al., 2007). Primers used to determine the presence of the F1534C mutation were: wild allele 1534P: 5'-GCG GGC TCT ACT TTG TGT TCT TCA TCA TAT T -3', mutant allele 1534C: 5'-GCG GGC AGG GCT CTT CTT TGT GTT CTT CAT CAT GTG -3', and a common one for both alleles: 5'-TCT GCT CGT TGA AGT TGT CGA T -3" (Harris et al., 2010).

All samples included positive controls for genotypes 1016 Val/Val, Val/Ile and Ile/Ile and 1534 Phe/Phe, Phe/Cys and Cys/Cys extracted from the DNA of the lines of *S. aegypti*: SS (Rock strain), RR (Rock-*kdr* strain) and RS (an equimolar mix or Rock and Rock-*kdr*). Rockefeller strain was obtained from the Laboratory of Physiology and Control of Arthropod Vector, at the Oswaldo Cruz Foundation, and has been kept in LAMFIC2 at UFPR at a controlled temperature of 25 ± 3ºC, 70% of RH, and under natural light. The AS-PCR amplifications were evaluated in 10% acrylamide gel and stained with Safer dye (Kasvi: 6x); through these readings, the genotypic and allelic frequencies were calculated, initially for each mutation and later as linked sites.

The analyses of the linked sites were performed following Linss et al. (2014). Hardy-Weinberg Equilibrium (HWE) was estimated for the sites linked by classical equation, and the null equilibrium hypothesis was verified by the chi-square test with one or three degrees of freedom when three or six genotypes, respectively, were evidenced. According to the alleles, the genotypes were named SS, SR1, SR2, R1R1, R1R2, and R2R2 (Linss et al., 2014; Macoris et al., 2018)

Molecular analyses in the ND4 gene

Amplification of the gene segment expressing subunit 4 of the mitochondrial enzyme NADH dehydrogenase was performed following the protocol of Twerdochlib et al. (2012). Primers used in the amplification of the ND4 gene segment were universal ND4R primers: 5'-ATT GCC TAA GGC TCA TGT AG-3' and a reverse primer NDAR: 5'-TCG GCT TCC TAG TCG TTC AT-3 (Gorrochotegui-Escalante et al., 2002). Sequencing was carried out according to Twerdochlib et al. (2012) protocol. The sequences were analyzed in Staden software version 1.5 and the alignment was performed in the Geneious software (Kearse et al., 2012), using the ClustalW tool (Thompson et al., 1994), and chromas version 2.1 was used for the sequence check (Goodstadt and Ponting, 2001). The obtained sequences were compared with those available in GenBank, using the tblastx tool to confirm the amplified fragment.

The genealogy among haplotypes was inferred by constructing of a network of haplotypes, which was elaborated with the aid of the TCS program version 1.21 (Clement et al., 2000). To estimate gene flow among the populations analyzed, a comparison was made with the haplotypes available in America, published by Gonçalves da Silva et al. (2012); these haplotypes are free of nuclear mitochondrial pseudogenes (NUMTs). To reduce the error caused by NUMTs in the samples, haplotypes were compared with a list of NUMTs verified by Black IV and Bernhardt (2009) and Hlaing et al. (2009). The NUMTs found were removed from the analysis. In addition, a phylogenetic analysis was performed to determine the genetic affinity of the populations of *A. aegypti* of this study with the sequences published by Gonçalves da Silva et al. (2012). In this analysis, all indels were considered. The resulting alignment was analyzed using the jModelTest program with the Akaike information criterion (AIC) to determine the most appropriate nucleotide evolution model (Darriba et al., 2012). After selecting the evolutionary model, a phylogenetic tree was constructed using Mega 6.1 program (Tamura et al., 2007). *Aedes albopictus* (Skuse, 1894) (GenBank # EF153761) was used as an external group.

Genetic diversity and neutrality tests were calculated in the DnaSP program, version 5.0 (Darriba et al., 2012). Molecular variation analysis (AMOVA) was performed using the Arlequin version 3.5 program (Librado and Rozas, 2009). The structure of the populations was verified by the fixation index (F_{ST}, Wright 1921) and the estimated gene flow (Nm), which was obtained using the Arlequin version 3.5 program (Excoffier and Lischer, 2010), and followed by the correction of Bonferroni (Rice, 1989). The Mantel test was used to estimate the correlation between genetics (F_{ST}) and geographic distance (km). Genetic isolation by distance was tested with the GenAlEx6 program (Peakall and Smouse, 2012). The geographic distance (km) was obtained through Google Earth 6.0.

Results

kdr mutations V1016I and F1534C were studied in 345 individuals of *A. aegypti*. Na_vS, Na_vR1 and Na_vR2 alleles were present in all studied locations, except the Na_vR3 alleles which were undetected. Allele frequency for NavR2 was 55%; the percentage of genotypic frequency was 22% for genotypes with potential for R1R2 resistance, 30% for R2R2, and 3% for SS genotype. Fig. 1 depicts the distribution of allelic frequencies related to the studied places.

The genotypic frequencies in most localities showed the predominance of SR2 and R2R2 genotypes, while the SS genotype was less frequent. The analyses by regions displayed distinction in the genotyping, being possible to differentiate the values that were not evident when the analysis was done by municipality. The analyses of the regions of each municipality exhibited a specificity resistance status; for instance, mosquitoes from the PE region, from the municipality of Alvorada do Sul was the only region to present the SS genotype; PE mosquitoes also had the lowest frequency values of R1R2 and R2R2 genotypes in this municipality. Alvorada do Sul is considered a rural adjacent municipality. Table 1 shows the genotypic frequencies of each locality, its regions and the Hardy-Weinberg Equilibrium.

The genetic variability was evaluated considering an amplification product of 257 bp with 44 polymorphic sites and 213 monomorphic sites. The analysis of 120 mosquitoes for the ND4 gene showed the existence of 40 haplotypes without NUMTs. The total relative frequency of the 40 haplotypes in the six populations of *A. aegypti* and the relative frequency of haplotypes in each population are shown in Table 2.

The H1 haplotype was the most frequent, representing 57.5% of the total haplotypes and was detected in all populations, except in Alvorada do Sul. The highest number of haplotypes was identified in Nova Londrina, with 15 haplotypes, followed by Alvorada do Sul with 12 and Marilena with 9 haplotypes; the other municipalities showed values lower than 5. Haplotypes were distinguished by 22 transitions: 11 a ↔ G (sites 23, 28, 45, 90, 148, 183, 185, 187, 208, 250, and 251), and 11 C ↔ T (sites 6, 11, 12, 13, 14, 21, 22, 130, 169, 180, and 238). In addition, it was observed 27 transversions: 6 A ↔ C (sites 8, 15, 23, 60, 139, and 252), 10 A ↔T (sites 9, 16, 17, 18, 19, 70, 178, 246, 249, and 256), 8 G ↔ T (sites 20, 22, 26, 27, 98, 99, 148, and 239), and 3 G ↔ C (sites 20, 64, and 139). The haplotypes (H) found in this study were similar to H1, H6, and H11, previously observed in Mexico, North America, Brazilian Amazon, Southeastern of Brazil, Peru, Mexico, and North America, respectively (Fig. 2).

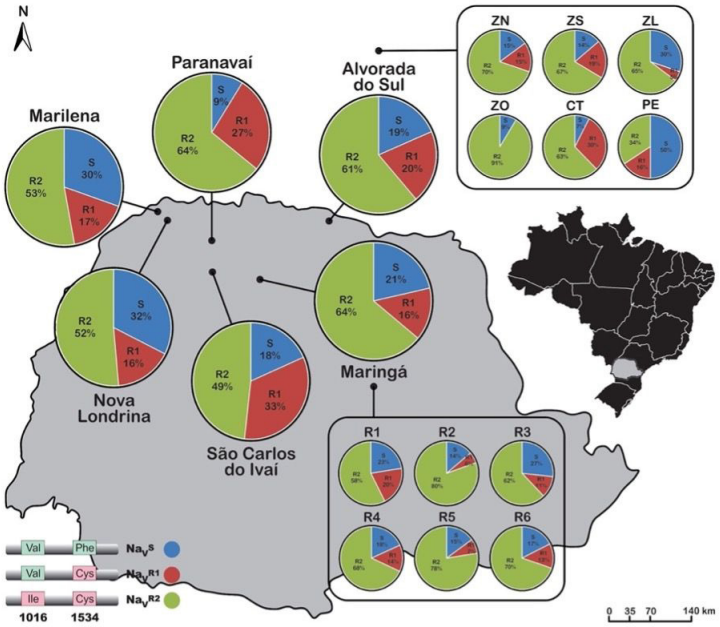

Figure 1 Distribution of the *kdr* alleles in *Aedes aegypti* populations for each Paraná locality. The state is detached, showing its multiple cities of collection.

Table 2
Haplotype frequencies in six *Aedes aegypti* populations from Paraná research localities.

Haplotype	AS	MR	MG	NL	PV	SC	Total	(%)
H1	0.0	3.0	12.0	3.0	26.0	25.0	69.0	57.5
H2			4.0				4.0	3.33
H3	2.0	1.0					3.0	2.50
H4				2.0			2.0	1.66
H5	1.0			1.0			2.0	1.66
H6		2.0					2.0	1.66
H7	1.0	1.0					2.0	1.66
H8	1.0			1.0			2.0	1.66
H9				2.0			2.0	1.66
H10		2.0					2.0	1.66
H11				1.0			1.0	0.83
H12				1.0			1.0	0.83
H13			1.0				1.0	0.83
H14	1.0						1.0	0.83
H15	1.0						1.0	0.83
H16	1.0						1.0	0.83
H17	1.0						1.0	0.83
H18		1.0					1.0	0.83
H19				1.0			1.0	0.83
H20				1.0			1.0	0.83
H 21	1.0						1.0	0.83
H22				1.0			1.0	0.83
H23				1.0			1.0	0.83
H24				1.0			1.0	0.83
H25				1.0			1.0	0.83
H26				1.0			1.0	0.83
H27				1.0			1.0	0.83
H28		1.0					1.0	0.83
H29	1.0						1.0	0.83
H30		1.0					1.0	0.83
H31					1.0		1.0	0.83
H32			1.0				1.0	0.83
H33						1.0	1.0	0.83
H34		1.0					1.0	0.83
H35	1.0						1.0	0.83
H36	1.0						1.0	0.83
H37					1.0		1.0	0.83
H38						1.0	1.0	0.83
H39						1.0	1.0	0.83
H40						1.0	1.0	0.83

The haplotypic diversity (h) of the populations of Paraná was h= 0.301, the nucleotide diversity (π) was π = 0.005, and the mean number of nucleotide differences (k) was k = 0.655. The neutrality tests of Tajima's D (-1.61663), Fu and Li's D (-0.08608), Fu and Li's F (-0.74613), and Fu and Li's Fs (-0.74613) were not significant for this sample ($p < 0.05$), indicating that they are in accordance with the neutral model of mutations. Molecular variance analysis (AMOVA) supported that genetic variation was higher within populations (86.28%), while among populations it was low; in addition, the analysis was significant ($F_{ST} = 0.13$; $p < 0.05$) supporting the hypothesis of genetic structuring. Genetic differentiation, represented by the peer-to-peer values of F_{ST} and nm values (number of migrants per generation) referring to the six populations of *A. aegypti* after Bonferroni correction are shown in Table 3.

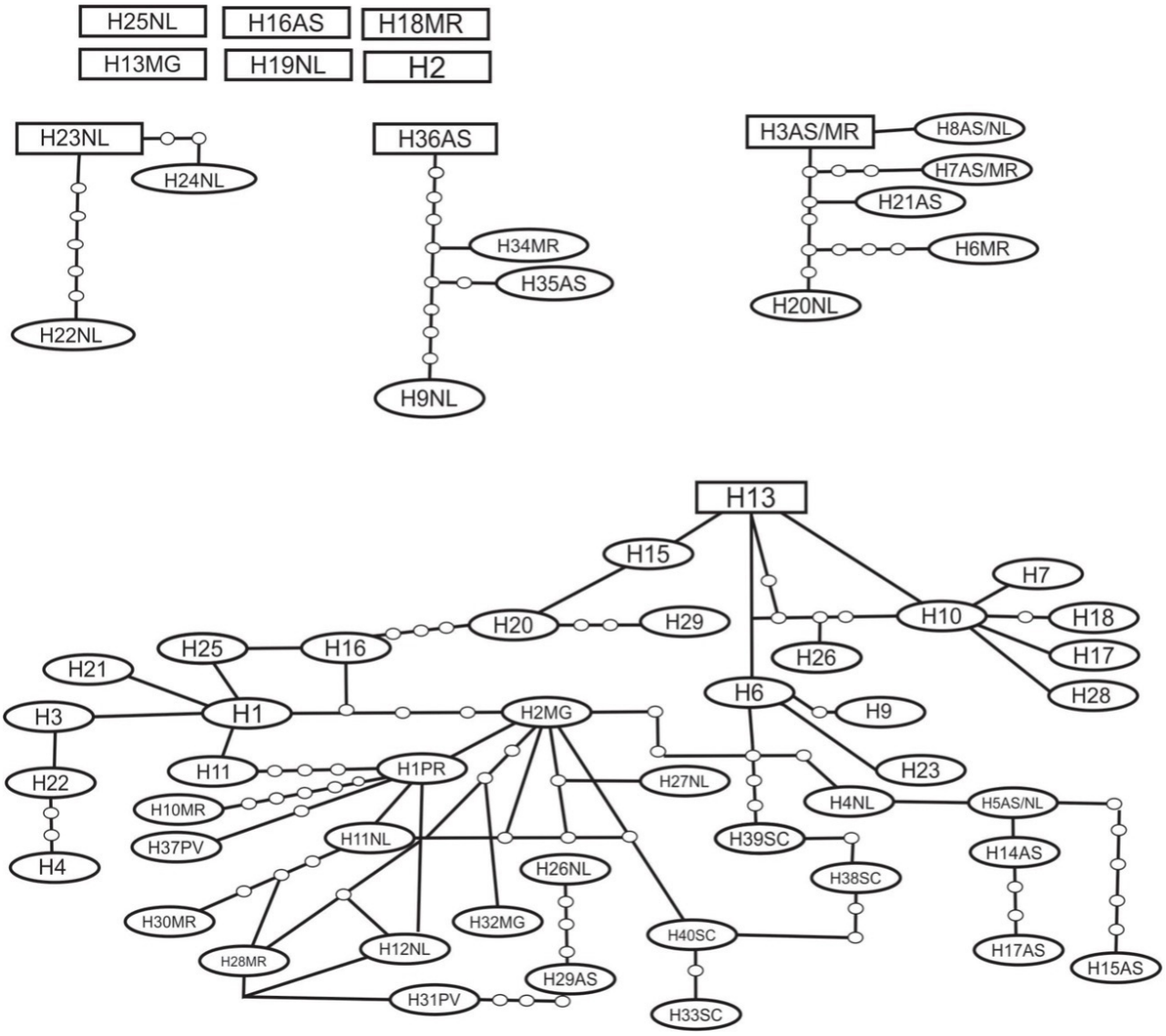

Figure 2 Haplotype network of ND4 gene of *Aedes aegypti* populations of the six minicipalities of Paraná and others from America (Gonçalves da Silva et al., 2012). The mosquitoes referring to this analysis were renamed with PR next to the haplotype number (ex: H1PR), to differentiate from the haplotypes (H) found by Gonçalves da Silva et al. (2012). The rectangle represents the ancestral haplotype. The smaller circles connecting the identified haplotypes correspond to the non-sampled haplotypes (missing haplotypes) and classified as intermediaries.

Table 3
Genetic distances (F_{st} values) and effective number of migrants (Nm), above and below the diagonal, respectively.

	AS	MR	MG	NL	PV	SC
AS	/	0.018	0.000	0.099*	0.000	0.000
MR	3.39 (185)	/	0.027	0.495*	0.000	0.000
MG	1.91 (100)	4.60 (130)	/	0.108*	0.009	0.027
NL	15.05 (179)	inf (7)	9.23 (130)	/	0.000	0.000
PV	0.73 (129)	1.24 (71)	8.65 (66)	2.69 (64)	/	0.000
SC	0.72 (139)	1.13 (88)	11.04 (57)	2.45 (81)	7.50 (26)	/

*Inf. = infinity; geographic distances (km) are in parentheses. * p > 0.05.*

The hypothesis of isolation by distance according to the correlation coefficient of the Mantel test revealed the existence of correlation between genetic distance (F_{ST}) and geographic distance (km; $R^2 = 0.0691$; $p < 0.05$). The results of the jModelTest program suggest that the best nucleotide evolution model for our data is the Neighbor-Joining model, following the Tamura-Nei genetic distance model. Fig. 3 presents the phylogenetic relationship indicating four large clades, the first containing the H1 haplotype, where most haplotypes were found.

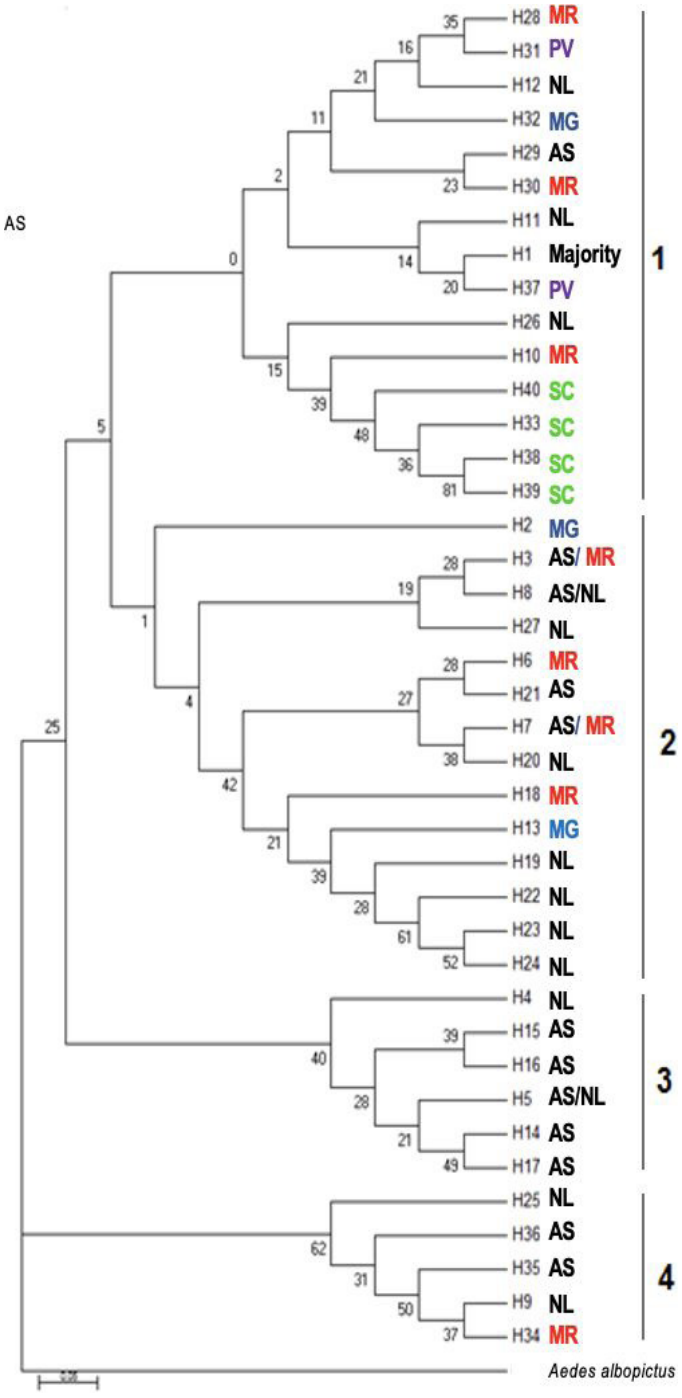

Figure 3 Dendrogram of the 40 haplotypes of *Aedes aegypti* divided into four groups. Neighbor-joining (NJ) tree of *A. aegypti* haplotypes using the Tamura-Nei parameter genetic distance model. Bootstrap values are marked under the respective nodes. *S. albopictus* was considered as external group. AS - Alvorada do Sul; MR - Marilena; MG -Maringá, NL - Nova Londrina; PV - Paranavaí; SC - São Carlos do Ivaí.

Discussion

In the current study, alleles Na_v S, NavR1 and NavR2 that confer resistance to PYs were identified in all populations of *A. aegypti* analyzed. Mutant alleles 1016I + 1534C were documented in more than half of the studied populations (55%), while R1R2, which indicates the population's potential to become resistant, was observed in 22% of the studied populations. Despite the fact that the Nav R1 and Nav R2 alleles are found in the same region of the sodium channel, both independently and at different levels confer the mosquito resistance to pyrethroids. This has been demonstrated from bioassays and electrophysiological analyses (Chen et al., 2020). The higher frequency of Nav R1 and Nav R2 alleles observed in this study may be related to the high and constant use of PYs to control vector populations, promoting increased selection pressure for these alleles (Chen et al., 2020; Vera-Maloof et al., 2020). These alleles have also been reported for other Brazilian populations (Linss et al., 2014; Chapadense et al., 2015; Macoris et al., 2018).

The presence of polymorphism related to the high frequencies of mutant alleles observed in currently studied populations concerns if the selection pressure is constant because of the fixation of the polymorphism, as previously observed in other Brazilian populations (Martins et al., 2009). When considering the current use of household products based on PYs (Martins et al., 2009; Ranson et al., 2010), the vulnerability to selection pressure of the mosquitoes from Paraná is high, as also observed in Portugal (Seixas et al., 2013), Venezuela (Alvarez et al., 2015), Colombia (Aguirre-Obando et al., 2015), Haiti (McAllister et al., 2012), and Mexico (Lopez-Monroy et al., 2018). On the other hand, the SS genotype, which is related to the wild status of *S. aegypti*, had a low frequency (3%). This means that compounds containing pyrethroids could effectively control only a few adults from the studied populations. Due to the resistance development, these results confirm the negative effects of the inappropriate use of the same active compound to control a target organism over the time. Similarly, our data indicate the need to create different molecules for vector control, and be efficient and safe for other organisms and the environment.

The genetic diversity values were low for Paraná populations ($h = 0.30$ and $\pi = 0.005$) when compared with the ones previously observed in this local ($h = 0.70$ and $\pi = 0.015$), which include three of the currently studied municipalities (Maringá, Nova Londrina and Paranavaí). The low genetic diversity indicates a connection between the reduction of genetic diversity over time (Twerdochlib et al., 2012) with the selection pressure caused by the insecticide exposure of the vector population (Herrera et al., 2006). The possible exchange of individuals of *A. aegypti* resistant to pyrethroids, mainly between Londrina and Maringá, and between the municipalities with São Paulo due to proximity or commercial interests, respectively, did not influence the genetic diversity observed in this work. Low values of nucleotide diversity ($\pi = 0.005$) have also been reported in populations of Bolivia ($\pi = 0.001$) (Paupy et al., 2012), and Thailand ($\pi = 0.008$) (Bosio et al., 2005). Nevertheless, high genetic diversity has been reported in mosquito populations that have been exposed continuously to a specific chemical compound, as reported in other populations from Brazil ($\pi = 0.017$ and $\pi = 0.011$) (Paduan and Ribolla, 2008; Lima Júnior and Scarpassa, 2009), México ($\pi = 0.014$) (Gorrochotegui-Escalante et al., 2002), and Venezuela ($\pi = 0.020$) (Herrera et al., 2006). The low genetic diversity in the present study indicated a relatively high gene flow between populations, a characteristic of expanded populations.

The current study showed that populations presented different mitochondrial haplotypes being a group I the most frequent one; this finding agrees with other studies (Gorrochotegui-Escalante et al., 2002; Herrera et al., 2006; Bracco et al., 2007; Paduan and Ribolla, 2008; Twerdochlib et al., 2012). Considering the haplotype network of

American populations (Gonçalves da Silva et al., 2012) along with the haplotypes of the six municipalities of Paraná, the presence of distinct groups including isolated haplotypes, could be noticed. In addition, a connection with the populations from Brazilian Amazon, Southeastern Brazil, Peru, Mexico, and North America could be noticed. In Brazil, two large genetic groups were reported, one descended from Venezuela and probably other countries from North America and another from the Caribbean (Monteiro et al., 2014).

Conclusion

To summarize, our results indicate low genetic diversity and gene flow in the populations of *A. aegypti*, as well as the occurrence in all populations of the Nav S, NavR1, and NavR2 alleles that confer resistance to PYs. These results indicate the need to monitor the frequency of resistance-related mutations to improve vector control programs' efficiency in some Paraná municipalities.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

MALF: Conceptualization, Methodology, Formal analysis, Data curation, Writing- Original draft preparation, Writing- Reviewing and Editing. OAAO: Methodology, Formal analysis. ALT: Conceptualization Methodology, Formal analysis. AMPC: Methodology, Data curation, Writing- Original draft preparation, Writing- Reviewing and Editing. MANS: Conceptualization, Methodology, Formal analysis, Data curation, Writing- Original draft preparation, Writing- Reviewing and Editing.

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