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Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in *Aedes aegypti* populations from Jacarezinho (Brazil) after a Dengue Outbreak

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ABSTRACT

After a dengue outbreak, the knowledge on the extent, distribution and mechanisms of insecticide resistance is essential for successful insecticide-based dengue control interventions. Therefore, we evaluated the potential changes to insecticide resistance in natural *Aedes aegypti* populations to Organophosphates (OP) and Pyrethroids (PY) after chemical vector control interventions. After a Dengue outbreak in 2010, *A. aegypti* mosquitoes from the urban area of Jacarezinho (Paraná, Brazil) were collected in 2011 and 2012. Insecticide resistance to OP Temephos was assessed in 2011 and 2012 by dose–response bioassays adopting WHO-based protocols. Additionally, in both sampling, PY resistance was also investigated by the Val1016Ile mutation genotyping. In 2011, a random collection of mosquitoes was carried out; while in 2012, the urban area was divided into four regions where mosquitoes were sampled randomly. Bioassays conducted with larvae in 2011 ($82 \pm 10\%$; $RR_{95} = 3.6$) and 2012 ($95 \pm 3\%$; $RR_{95} = 2.5$) indicated an incipient altered susceptibility to Temephos. On the other hand, the Val1016Ile mutation analysis in 2011, presented frequencies of the $1016Ile^{kdr}$ allele equal to 80%. Nevertheless, in 2012, when the urban area of Jacarezinho was analyzed as a single unit, the frequency of the mutant allele was 70%. Additionally, the distribution analysis of the Val1016Ile mutation in 2012 showed the mutant allele frequencies $\geq$60% in all regions. These outcomes indicated the necessity of developing alternative strategies such as insecticide rotations for delaying the evolution of resistance.

Introduction

Dengue, a viral disease transmitted by a mosquito, has the greatest epidemic potential in the world, and a negative impact on the economy and health of the population in urban areas. The mosquito *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) is the primary vector of dengue in the Americas and particularly in Brazil (WHO, 2013). In Brazil, *A. aegypti* is the main vector of dengue virus, which is also a vector for yellow fever and chikungunya viruses (Lourenço-de-Oliveira, 1994). Brazil is especially vulnerable to dengue epidemics, since almost 60% of dengue cases reported in Latin America from 2001 to 2011 were registered in the country (Dick et al., 2012). Currently, the entire country is endemic for dengue and the last outbreak in 2013 accounted for more than 1.3 million cases (OPS, 2013).

Studies have been conducted to develop a vaccine against dengue (Lu et al., 2013; Sun et al., 2013), but due to its complexity, the means of control still have a major influence on vector population. Control of *A. aegypti* primarily consists on the elimination of artificial and disposable water flooded larvae breeding sites and the application of insecticides (WHO, 2013). Different classes of insecticides have been successively used since 1950s, but most current control programmers are largely dependent on the use of organophosphates (OP) and pyrethroids (PY) (Hemingway and Ranson, 2000; Shetty et al., 2015; Baldacchino et al., 2015). In recent decades, PY insecticides have played a major role in the control of adult *A. aegypti*, often used in combination with OP Temephos to control larvae development (Bisset et al., 2013; Nkya et al., 2013). However, PY and OP resistance in *A. aegypti* and other vector populations has compromised the effectiveness of these control programs (Linss et al., 2014; Saavedra-Rodrigues et al., 2014).

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Therefore, new promising insect control efforts are currently being evaluated such as biological alternatives or even transgenic insects and *Wolbachia* based strategies (Dutra et al., 2015; Olson and Franz, 2015).

In Brazil the OP Temephos has been used as a larvicide since 1967, with the introduction of PY Cypermethrin and Deltamethrin since 1999 to control of adults (Braga et al., 2004; Braga and Valle, 2007). In Brazil, the first cases of resistance to OP Temephos and PY were recorded in 1999 (Goiás and São Paulo states) and 2001 (Alagoas, Sergipe and Rio de Janeiro states) (Macoris et al., 1995; Pereira Da-Cunha et al., 2005). Currently, resistance to OP and PY has been developed throughout the country (Linss et al., 2014; Da Silva et al., 2015). However, in areas with confirmation of resistance to OP Temephos, insect growth regulators larvicidal (Benzoilfenylureas; i.e., Diflubenzuron and Novaluron) are used; these are chemical insecticides which act on the synthesis of chitin by altering growth and development of insects (Borges et al., 2012).

Although OP and PY have different action mechanisms, both act in the nervous system causing death to the insect (IRAC, 2010). OP and carbamate insecticides affect nerve synapses through the neuro-transmitter acetylcholinesterase, while PY and the organochlorines (DDT) aim the sodium channels of the nerve sheath, producing an effect similar to a knockdown. Metabolic resistance and target site insensitivity, represent the two major forms of OP and PY resistance (Soderlund and Knipple, 1999, 2003). Metabolic resistance is conferred by alterations in the levels or activities of detoxification enzymes, predominately esterases (EST), the multi-function oxidases (MFO), and the glutathione-S transferases (GST) super-families (Montella et al., 2012). Structural changes in the insecticide target site, in the voltage-gated channel sodium channel (Na_v) can lower the affinity for the insecticide (Liu, 2015). Cross-resistance between PY and DDT is frequently due to mutations in the Na_v proteins (Dong et al., 2014). Studies have suggested that mutations in the Na_v, the target site for PY and DDT, may be playing a role in PY resistance (Rinkevich et al., 2015). Na_v, is a transmembrane protein present in the neuronal axons and is composed of four homologous domains (I–IV), each with six hydrophobic segments (S1–S6) (Catterall, 2000). PY resistance, known as knockdown resistance (kdr) is associated with changes in the target site of the insecticide caused by mutations in the nucleotide sequence of Na_v (Dong et al., 2014). Kdr mutations in the Na_v (associated or not with PY resistance) have been observed in a range of insects, including *A. aegypti* (Saavedra-Rodriguez et al., 2007).

In insects, most resistance associated mutations are found in the IIS6 and IIIS6 of the Na_v protein (Brito et al., 2013). In *A. aegypti*, twelve non-synonymous mutations at nine different loci have been reported (Saavedra-Rodriguez et al., 2007; Chang et al., 2009; Harris et al., 2010; Linss et al., 2014; Kushwah et al., 2015), amongst which the kdr mutations: Ile1011Val/Met and Val1016Ile in the IIS6 segment and Phe1534Cys in the IIIS6 segment, are most commonly reported as contributing to PY resistance (Dong et al., 2014). However, the role in PY resistance of kdr mutation Ile1011Val/Met remains to be elucidated (Martins et al., 2009a). Reports of field and laboratory studies from North and South America reveal a fast dissemination of PY resistance and a drastic increase in the rates of the Val1016Ile mutation in *A. aegypti* populations (Rodpradit et al., 2005; Strode et al., 2008; García et al., 2009; Martins et al., 2009a; Lumjuan et al., 2011; Marcombe et al., 2012; Saavedra-Rodrigues et al., 2012). Nevertheless, a recent study suggests the co-occurrence of two kdr alleles are contributing to PY resistance: substitutions restricted to the 1534 position (Na_v^{R1}) or simultaneous substitutions in both 1016 and 1534 sites (Na_v^{R2}) (Linss et al., 2014).

Early studies on *A. aegypti* populations carried out in Latin America have shown the presence of mutations Ile1011Met, Val1016Ile and Phe1534Cys (Brengues et al., 2003; García et al., 2009; Martins et al., 2009b; Linss et al., 2014; Aguirre-Obando et al., 2015). On the other hand, studies carried out in Brazil, showed the Ile1011Met substitution was found in *A. aegypti* population from Belém, PA, with low sensitivity to PY as measured by an electrophysiological assay (Brengues et al., 2003). Martins et al. (2009b) reported high frequencies of the Val1016Ile mutation in five populations of *A. aegypti* from Brazil that were resistant to deltamethrin. In 2013, the Phe1534Cys substitution was also found in Brazilian *A. aegypti* populations (Seixas et al., 2013). Currently, the mutations Val1016Ile and Phe1534Cys have spread throughout Brazil (Linss et al., 2014).

In Brazil, Jacarezinho town is located in the northern of Paraná state. It borders municipalities with populations ranging from 10 to 45 thousand inhabitants (IBGE, 2015). Within Paraná, Jacarezinho is far from the influence of the largest urban centers (i.e., Londrina, Maringá and Foz do Iguaçu), where have been registered the occurrence of large dengue outbreaks; these urban centers are normally considered as vector scattering centers (Duque et al., 2010).

In 2010, the Paraná state presented a total of 28.511 indigenous cases of dengue. From that totality, Jacarezinho town, showed a significant increase in the number of indigenous cases of dengue, ranking second in the state (3.892) after Londrina (7.593), which led to declare the state of emergency for dengue cases. These events led to a significant increase in chemical vector control in Jacarezinho (Secretaria do Estado da Saúde/Estado do Paraná, 2010).

In 2011, there was a reduction in the number of dengue indigenous cases in Jacarezinho. This reduction was considered low (1.0), when compared to the large urban centers like Londrina (84), Foz do Iguaçu (133) and Maringá (76) (Secretaria do Estado da Saúde/Estado do Paraná, 2011). Here, we evaluated how the insecticide resistance of *A. aegypti* populations to PY and OP Temephos may be influenced by the intensification of vector chemical control, which, in subsequent years was marked by a significant number of dengue cases.

Material and methods

Sampling

Between 2011 and 2012, *A. aegypti* samples were taken from the urban area of Jacarezinho town (23°09′38″ S, 49°58′10″ W), Brazil (Fig. 1). Jacarezinho is located in the northern region of the Paraná state (Brazil; Fig. 1a), with a population of 40,221 inhabitants (11% in rural areas and 89% in urban areas), an area of 602,529 km^2 and a Municipal Human Development Index of 0.743. Jacarezinho is crossed by two main roads, one federal highway (BR-153) and two state highways (PR-431 and PR-515) (Fig. 1b). The BR-153, goes through Jacarezinho from north (São Paulo, SP) to south (Santo Antônio, PR); besides, this is the fourth major highway from Brazil since it goes over 4.355 km from Pará to Rio Grande do Sul states. On the other hand, the PR-431 and PR-515 goes through Jacarezinho from east (Cambará and Barra do Jacaré, PR) to west (Riberão Claro, PR) (Fig. 1b; IBGE, 2010). The samples were collected by the *Secretaria Municipal de Jacarezinho* using ovitraps (Fay and Eliason, 1966). Each ovitrap was installed each 100 m away from each other in the peridomestic area. The collected *Aedes* sp eggs were kept under controlled conditions (25 ± 10 °C, humidity 80 ± 10% and photoperiod 12:12 h) until the adults emerged.

To analyze the frequency of the Val1016Ile mutation in 2011 and 2012, two sampling forms were carried out. In 2011, the urban area of Jacarezinho was considered as an independent unit of ovitraps location. Therefore, the sampling carried out in 2011 allowed the

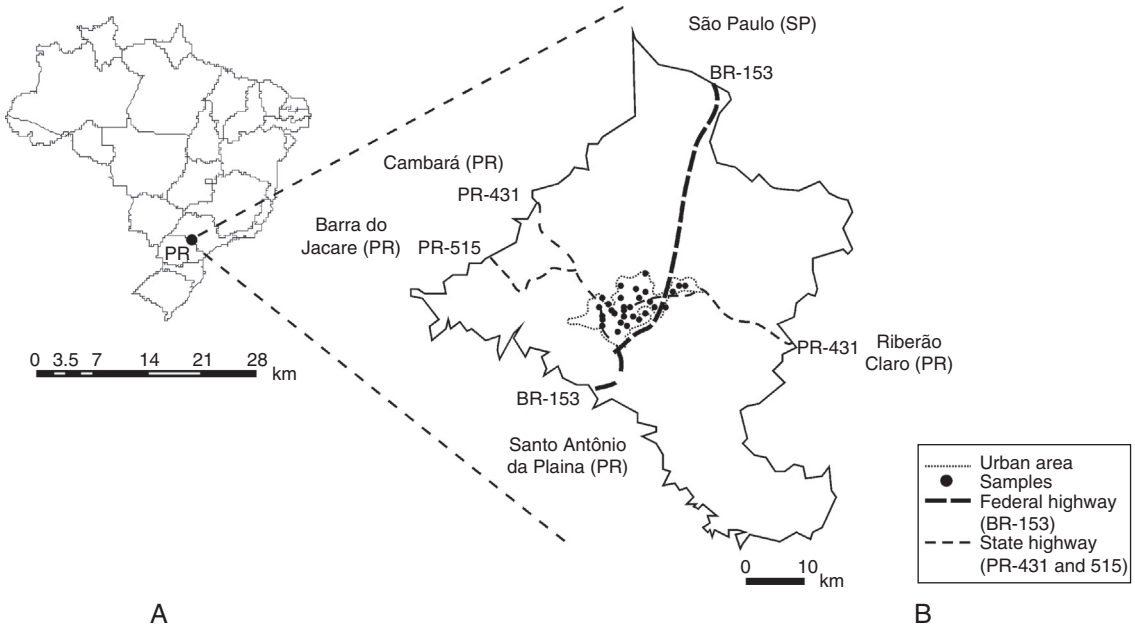

Fig. 1. Location of Jacarezinho town (PR = Paraná state) in Brazil (A). Jacarezinho map shows the collection sites in the urban area used for the analysis of the Val1016Ile mutation in 2011 and 2012. Additionally, the main roads that cross Jacarezinho are presented (B).

formation of the Jacarezinho 2011 strain, in which 50 adults of *A. aegypti* were randomly taken.

In the 2012 sampling, the Jacarezinho urban area was divided into four regions (RI–RIV; Fig. 2). These collection sites were also, the same used for Jacarezinho 2011 sampling. The division into regions was used in order to check the presence of distribution of the kdr mutation in the urban area. Each region gave birth to an *A. aegypti* strain, maintained under laboratory conditions, from which 15 adults of *A. aegypti* were randomly collected. After collecting the adults, four *A. aegypti* strains were pooled into one, forming the Jacarezinho 2012 strain.

In 2011 and 2012 sampling, recently emerged adults were collected. In both samples, the mosquitoes were individually placed in absolute ethanol (99.5%) and stored in a freezer at −20 °C. The remaining F_0 adults from Jacarezinho 2011 and 2012 strains were used to produce the F_1 *A. aegypti* generation. *A. aegypti* F_1 larvae from Jacarezinho 2011 and 2012 were used as the source in bioassays to determine Temephos susceptibility. Adults from Jacarezinho 2011 and 2012 strains were fed on a 10% honey solution and blood meals that were provided by mice (*Mus muscullus* Swiss) twice a week to induce oviposition (Certificate no.719; Animal Ethics Committee, Federal University of Paraná).

Bioassays with Temephos insecticide

The larval bioassays were conducted according to WHO guidelines using F_1 generation larvae and the insecticide Temephos pestanal (Sigma–Aldrich; 250 mg 97.5%) (WHO, 1998). Bioassays were calibrated with Rockefeller, a susceptible strain of *A. aegypti* (Centers for Disease Control, CDC), using a diagnostic concentration of 0.0162 ppm Temephos (as it was previously determined by our laboratory). This is twice the LC_{99} (lethal concentration that kills 99% of the larvae) of the susceptible strain. Four samples were used in each test. Each sample consisted of 20 larvae, late third instar, and fourth instar of *A. aegypti*, in 100 mL of purified water. As population controls, four replicates using ethanol as a solvent were tested. Each bioassay was repeated four times on different days. The results from larvae were expressed as mortality rates 24 h after exposure to Temephos. The following criteria proposed by WHO (1998) guidelines, were adopted to classify populations susceptibility status: susceptible (percentage of mortality > 98%), an incipient altered susceptibility (80–98%) or resistant (<80%).

Dose–response bioassays followed WHO procedure in order to determine larval susceptibility to Temephos (WHO, 1981). In these experiments, third-instar or initial fourth-instar larvae were exposed to 10 concentrations (including the diagnostic concentration) of the insecticide to determine larval mortality rate between 5 and 95%. The concentrations tested ranging between 0.0042 and 0.0312 ppm. The concentration range between them was of 0.003 ppm. For each concentration and for the control, four replicates of 20 larvae, totaling 880 larvae per experiment, were tested. Each concentration was performed in 100 mL of purified water. All tests were repeated four times at different days. Larval mortality was checked 24 h after exposure.

Mortality data (expressed in number of dead specimens per dose) was applied to calculate lethal concentrations to 50 and 95% (LC_{50} and LC_{95}) of exposed individuals, and analyzed by the log-probit method of Finney (1971) using the Probit software by Raymond (1985). Resistance ratios (RR_{50} and RR_{95}) were obtained by dividing the lethal concentration of the population by the equivalent lethal concentration of the Rockefeller population.

DNA extraction and molecular analysis of the Val1016Ile mutation

DNA extraction followed Bona et al. (2012). Individual mosquitoes were placed in 1.5 mL microcentrifuge tubes, homogeneized with 160 μL of lysis buffer (Tris–HCL, pH 8.0 200 mM, NaCl 2.0 M, EDTA, pH 8.0 70 mM and sodium dodecyl sulfate 1%) and incubated at 60 °C for 30 min followed by addition of 50 μL of chloroform: isoamilic (24:1) The mixture was centrifuged at 13,000 × *g* for 15 min. The DNA present in the supernatant was precipitated by ethanol. The final DNA pellet was re-suspended in 20 μL of TE buffer (Tris–HCl 10 mM, EDTA, pH 8.0 1.0 mM) and stored at −20 °C.

In this study, we indirectly genotyped the Na_v^{R2} allele. Individual mosquitoes from each sampling were genotyped at the 1016 position from genomic DNA using allele-specific PCR (AS-PCR). We used three primers to determine the presence of the mutation

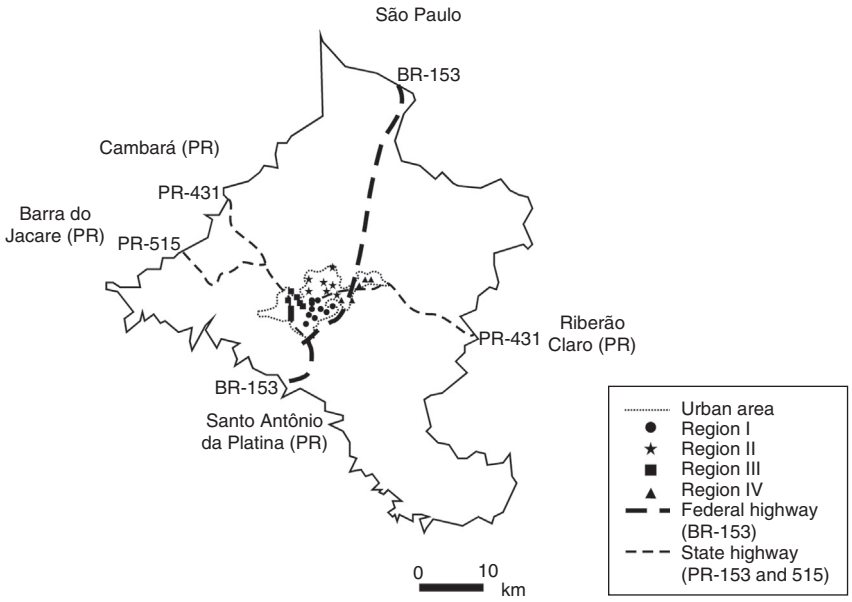

Fig. 2. Jacarezinho map shows the collection sites by regions in the urban area used for the analysis of the Val1016Ile mutation in 2012. Additionally, the main roads that cross Jacarezinho are presented.

Val1016Ile, one for the allele 1016Val: 5′-GCG GGC AGG GCG GCG GGG GCG GGG CCA CAA ATTGTT TCC CAC CCG CAC CGG-3′, one for allele 1016Ile: 5′-GCG GGC ACA AAT TGT TTC CCA CCC GCA CTG A-3′, and a third common for both alleles: 5′-GGA TGA ACC GAA ATT GGA CAA AAG C-3′. PCR was carried out with the GoTaq kit (Promega) containing 1.0 μL of genomic DNA, 0.24 μM of the primer common for both alleles and 0.12 μM of the primers 1016Val and 1016Ile. Complete reaction volume totaled 12.5 μL. Denaturing, annealing and extension conditions were respectively, 94 °C/30″, 62 °C/1″ and 72 °C/5″, in 30 cycles (Saavedra-Rodriguez et al., 2007; Martins et al., 2009b).

Amplified alleles were checked on 10% polyacrylamide gel. For collections carried out in 2011 and 2012, using the gel results, we calculated genotypic and allelic frequencies, and Hardy–Weinberg equilibrium (HWE) (Salman, 2007; Harth, 2008). For 2012 sampling, the genotype and allele frequencies, as well as the HWE were analyzed in two different ways: first, the urban area of Jacarezinho was considered and analyzed as a single unit; and second, it was analyzed by regions. Rockefeller strain, continuously reared in the laboratory as a standard for insecticide susceptibility, and life-history trait parameters, were used as reference for wild-type alleles (1016Val) for the Na_v gene.

Results

In 2011, a total of 83 ovitraps distributed in the urban area of Jacarezinho, resulted in 850 mosquitoes. On the other hand, in 2012, a total of 103 ovitraps that also distributed in the urban area of Jacarezinho, resulted in 1425 adults. Thus, in 2012, the distribution of *A. aegypti* by region (R) was: 255 RI, 157 RII, 450 RIII and 563 RIV. The average number of eggs per ovitrap in 2011 and 2012 were 10 and 13, respectively.

Diagnostic concentration (% mortality)

Bioassays with larvae showed an incipient altered susceptibility to OP Temephos in the *A. aegypti* populations (Mortality rate ± SD, n = sample size) from Jacarezinho 2011 (82% ± 10, n = 320) and 2012 (95% ± 3, n = 320).

Multiple concentrations (RR)

Dose–response bioassays showed resistance ratios (RR_{95}) greater than three in 2011 and lower than three in 2012. In general, the slope values of the studied *A. aegypti* populations were lower than those obtained from the Rockefeller strain, confirming their heterogeneity in comparison to the reference strain and the differences in their response to OP Temephos. The LC_{50} and LC_{95} of population studied are presented for comparison in Table 1.

Molecular analysis of the Val1016Ile mutation

In 2011, the analysis at position 1016, showed a frequency of the $1016Ile^{kdr}$ allele equal to 80%. In 2012, when the urban area of Jacarezinho was analyzed as a single unit, the frequency of the mutant allele was 70%. However, in 2012, when the Val1016Ile mutation was analyzed by regions from the urban area, the $1016Ile^{kdr}$ allele frequencies > 60%, were observed in all regions (Fig. 3).

The genotype frequency analysis of the Jacarezinho 2011 strain indicated that 61% had the homozygous recessive genotype (Ile/Ile), 33% heterozygous (Val/Ile) and 6% homozygous dominant (Val/Val).

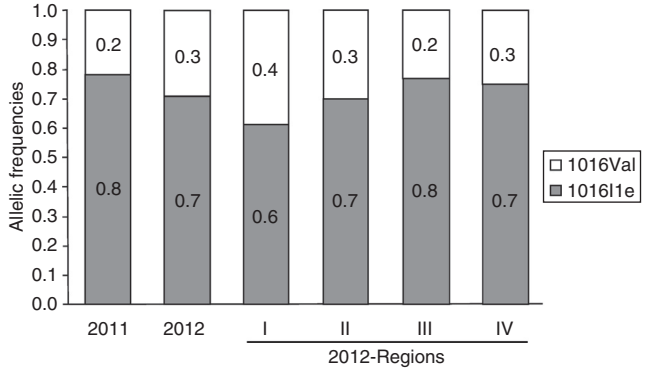

Fig. 3. Allelic frequencies of 1016Val and 1016Ile in the Na_v of *A. aegypti* populations from Jacarezinho in 2011 and 2012. Besides, the allelic frequencies of the Val1016Ile mutation by regions (Region I–IV) for 2012 are presented.

Table 1
Temephos susceptibility profile of 2011 and 2012 *A. aegypti* populations from Jacarezinho, showing the average for slopes, LC and RR.

Population	Year	*F*	*N*	Slope	LC (CI)		RR	
					50	95	50	95
Rockefeller	–	Fn	3520	5.0 ± 0.2	0.0034 (0.0032 ± 0.0036)	0.0063 (0.0059 ± 0.0067)	–	–
Jacarezinho	2011	F1	3200	4.7 ± 0.2	0.0103 (0.0099 ± 0.0107)	0.0228 (0.0218 ± 0.0240)	3.0	3.6
Jacarezinho	2012	F1	3200	4.3 ± 0.2	0.0066 (0.0063 ± 0.069)	0.0159 (0.0151 ± 0.0171)	1.9	2.5

F, filial generation; *N*, sample size; $CI_{(\alpha=0.05)}$, confidence interval.

Table 2
Genotypic frequency of the mutation Val1016Ile in the Na_V of *A. aegypti* from Jacarezinho for 2011 and 2012. Additionally, the genotypic frequencies of the Val1016Ile mutation by regions (Region I–IV) for 2012 are presented.

Year of collecting	Region	*n*	Genotypic frequency			
			Val/Val	Val/Ile	Ile/Ile	X^2 (df)[a]
2011	–	50	0.06	0.33	0.61	0.19 (1)
2012	–	60	0.00	0.59	0.41	9.86 (1)[a]
2012	I	15	0.00	0.79	0.21	5.90 (1)[a]
	II	15	0.00	0.60	0.40	2.76 (1)
	III	15	0.00	0.46	0.54	1.16 (1)
	IV	15	0.00	0.50	0.50	1.41 (1)
Total		110				

[a] Significant difference ($p < 0.05$).

For 2012 collection, when the vector population was analyzed as a single unit, 59% of the samples presented the heterozygous genotype (Val/Ile) and 41% the homozygous recessive genotype (Ile/Ile). There was not identified any homozygous dominant genotype (Val/Val). However, in 2012, when the genotype frequencies of the kdr mutation were analyzed by regions, the Ile/Ile genotype was >40% in most of the urban area regions. In only two out of six samplings the HWE assumption was rejected (Table 2).

Discussion

We found high frequency of $1016Ile^{kdr}$ allele, as well as an incipient altered susceptibility to OP Temephos throughout the urban area of Jacarezinho. Changes in the susceptibility status of *A. aegypti* populations to OP Temephos have been previously reported in Brazil (Carvalho et al., 2004; Macoris et al., 2007; Prophiro et al., 2011) and in Thailand (Jirakanjanakit et al., 2007), India (Sharma et al., 2004), Cambodia (Polson et al., 2001) and Venezuela (Mazzari and Georghio, 1995). These findings were probably due to reduced vector densities in the cold seasons, together with lower selective pressure due to less intense use of insecticides. However, resistance to OP Temephos has been developed throughout Brazil (Da Silva et al., 2015). According to WHO (1992), the *A. aegypti* populations that present susceptibility variation to Temephos should reassess the population susceptibility status, since Temephos pressure on larvae may generate cross-resistance to PY or even to other OP used in the control of the adult stages (Tikar et al., 2009). Besides, it was found that PY pressure on larvae and adult of *Culex quinquefasciatus* (a filariasis vector) under laboratory conditions may also generate cross-resistance to OP (Ramkumar and Shivakumar, 2015). Our results showed $RR_{95} > 3.0$ in *A. aegypti* populations from Jacarezinho in 2011, in consequence, the OP Temephos should be switched by another insecticide before Temephos resistance compromises operational control. In studies conducted with Brazilian *A. aegypti* populations, when the application of Temephos is interrupted in locations where $RR_{95} > 10$, OP resistance will only decline gradually and will require several years to begin using Temephos again (Montella et al., 2007).

In Jacarezinho, the presence of the $1016Ile^{kdr}$ allele (showing high frequency) may suffer an expansion as a result of actions control using PY insecticides. These findings could be explained due to since 2001 until 2009 the Brazilian Dengue Control Program employed PY in ultralow volume applications in several municipalities as part of the effort to control the dengue vector (Montella et al., 2007). In the last decade, the *A. aegypti* populations from Brazil and Mexico show a rapidly spread of the $1016Ile^{kdr}$ allele, simultaneously with the intensification of PY usage due to the emergence of dengue outbreaks (García et al., 2009; Linss et al., 2014). Although bioassays with adults to determine susceptibility to PY were not performed, the detection the Val1016Ile mutation in *A. aegypti* natural populations has dire consequences for the continued use of PY, since studies on selection pressure using PY insecticides and under laboratory conditions have documented the fixation of the $1016Ile^{kdr}$ allele after only five generations (Saavedra-Rodrigues et al., 2012). Therefore, enhanced surveillance for resistance should be a priority in localities where the $1016Ile^{kdr}$ allele is found, before new adaptive alleles can be selected for decreasing the deleterious effects of kdr (Brito et al., 2013).

Additionally, the distribution analysis of the $1016Ile^{kdr}$ allele was also found in high frequency in all regions. These outcomes suggest that the applications of PY used by the Brazilian vector control program as well as citizens (i.e., household insecticides, mosquito repellents and impregnated bed nets) are applied across the urban area. The evolution and spread of resistance to insecticides is a major concern for the control of all arthropod transmitted infections and *A. aegypti* is no exception (Vontas et al., 2012). Since *A. aegypti* is essentially an urban mosquito, it is constantly exposed to strong PY selection. Therefore, the $1016Ile^{kdr}$ allele has been rapidly selected and spread on many Latin America *A. aegypti* populations (Linss et al., 2014; Alvarez et al., 2015; Aguirre-Obando et al., 2015). Another possibility to explain the homogeneous spread of the $1016Ile^{kdr}$ allele across the urban area could be by the passive human transportation networks of mosquitoes, through the main highways of Jacarezinho. These means of transportation are globally known for being the main mode of long-distance dispersion (Gonçalves et al., 2012). Therefore, we suggest the execution of a genetic population analysis in order to establish this hypothesis.

Consequently, high frequency of the $1016Ile^{kdr}$ allele found usually comes with associated fitness costs, as previous work under laboratory conditions showed (Brito et al., 2013). Nevertheless, the fitness costs of the studied populations were not performed.

A stage-structured deterministic model parametrised (comprising five stages, and three genotypes (susceptible, heterozygous and resistant) associated to kdr mutations) for *A. aegypti* showed that in the absence of selective pressure of PY, the frequency of a costly resistance allele is expected to drop down. However, PY resistance decline can take, in some cases, decades even if fitness costs are not small (Schechtman and Souza, 2015); a trend also found when Temephos resistance is high (Montella et al., 2007). The rapid increase of the 1016Ile^{kdr} allele in natural *A. aegypti* populations emphasizes the need for developing alternative strategies such as insecticide rotations and mixtures to delay the evolution of resistance, a phenomenon that most likely encumbers dengue control programs (Maciel-de-Freitas et al., 2014). We conclude then, that the vector control actions must be supported in different approaches focusing mainly on prevention, since when they are focused in a single emergency situation method, without monitoring the susceptibility history, the result is the maintenance of the resistance to insecticides in the vector population.

Conflicts of interest

The authors declare no conflicts of interest.

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