Journal of the American Mosquito Control Association, 40(1):20–25, 2024
Copyright © 2024 by The American Mosquito Control Association, Inc.

INSECTICIDE SUSCEPTIBILITY AND *KDR* MUTATIONS IN *AEDES ALBOPICTUS* COLLECTED FROM SEVEN DISTRICTS OF GUANGYUAN CITY, NORTHERN SICHUAN, CHINA

QIONGYAO ZHAO,[1] YONGCHAO JIA,[1,3] XIAOQIANG LU,[1] YANCHUN LIU,[1] ZHONGYI YIN,[1] YANFANG ZHANG,[1] YU FU,[1] XING LUO,[1] ZICAI CHU[1] AND XINGHUI QIU[2,3]

ABSTRACT. The Asian tiger mosquito, *Aedes albopictus*, is an important vector of chikungunya, dengue, yellow fever, and Zika viruses. Vector control remains an important means for the prevention and control of vector-borne diseases. The development of insecticide resistance has become a serious threat to the efficacy of insecticide-based control programs. To understand the resistance status and the underlying genetic mechanism in mosquitoes in Guangyuan City of Sichuan Province, China, we investigated the susceptibility of *Ae. albopictus* to four commonly used insecticides. We found that all the examined populations were susceptible to malathion and propoxur. However, *Ae. albopictus* populations in Guangyuan showed a possible resistance to the two tested pyrethroids (beta-cypermethrin and deltamethrin). Notably, phenotypic resistance to deltamethrin was detected in 2 of the 7 populations. The potential of resistance to pyrethroids was confirmed by the presence of knockdown resistance (*kdr*) related mutations in the voltage-gated sodium channel. Four *kdr* mutations (V1016G, I1532T, F1534L, and F1534S) were identified to be present alone or in combination, and their distribution displayed significant spatial heterogeneity. These findings are helpful for making evidence-based mosquito control strategies and highlight the need to regularly monitor the dynamics of pyrethroid resistance in this city.

KEY WORDS *Aedes albopictus*, Guangyuan City, Sichuan Province, insecticide susceptibility, knockdown resistance, voltage-gated sodium channel

Downloaded from http://meridian.allenpress.com/jamca/article-pdf/40/1/20/3336206/i1943-6270-40-1-20.pdf by guest on 30 May 2024

INTRODUCTION

Aedes albopictus (Skuse), the Asian tiger mosquito, is a major disease vector that can transmit several important arboviruses including chikungunya, dengue, yellow fever, and Zika viruses (Gratz 2004). This species is one of the most common mosquito species in China (Liu et al. 2019). Currently, insecticide-based mosquito control remains an important means for the prevention of vector-borne diseases. However, continuous use of insecticides in the field or in domestic settings has led to the development of insecticide resistance in many *Ae. albopictus* populations (Marcombe et al. 2014, Xu et al. 2016, Moyes et al. 2017, Li et al. 2018), posing a serious threat to disease control. Understanding the status of insecticide resistance and the genetic mutations responsible for resistance is crucial to the successful establishment of *Ae. albopictus* control strategies.

Guangyuan City is located in the north of Sichuan Province of China with a total area of 16,319 square kilometers and a population of nearly 3 million. A recent survey showed that *Ae. albopictus* had a relatively high density (Luo et al. 2021) and cases of imported dengue fever have been reported in the recent decade. Moreover, a previous study showed that *Ae. albopictus* larvae displayed a low level of resistance (2.11-fold) in 2015 (Jia et al. 2018). These observations call for further studies on the status and trends of insecticide resistance in this dengue vector.

The present study is an effort to understand the current status of insecticide resistance to 4 insecticides recommended by the Chinese Center for Disease Control and Prevention in *Ae. albopictus* across the city of Guangyuan. In addition, the occurrence and frequency of the recognized pyrethroid resistance–conferring mutations in the voltage-gated sodium channel (VGSC) were also investigated.

MATERIALS AND METHODS

Aedes albopictus samples

Aedes albopictus larvae were collected during July to September 2020 from different sampling sites in 7 districts or counties of Guangyuan City of Sichuan Province (Fig. 1). The larvae caught from the same districts were pooled and reared to adults in the laboratory. The adults from the F1 or F2 progeny were used for susceptibility bioassays after morphological identification. The F0 *Ae. albopictus* adults were kept in absolute ethanol and stored at –20°C until use for DNA extraction.

Insecticide susceptibility bioassays

Insecticide susceptibility bioassays were carried out according to the World Health Organization (WHO) guidelines (WHO 2016) under the conditions of 25 ± 1°C and 70% RH. The 4 test insecticides, applied at the diagnostic dose to insecticide-impregnated papers, were 0.4% beta-cypermethrin (pyrethroid), 0.1% deltamethrin (pyrethroid), 0.05% propoxur (carbamate),

[1] Guanyuan Center for Disease Control and Prevention, No. 203 Binhe North Rode, Dongba District, Guangyuan, Sichuan 628040, China.
[2] Institute of Zoology, Chinese Academy of Sciences, 1-5 Beichen West Road, Chaoyang District, Beijing 100101, China.
[3] To whom correspondence should be directed.

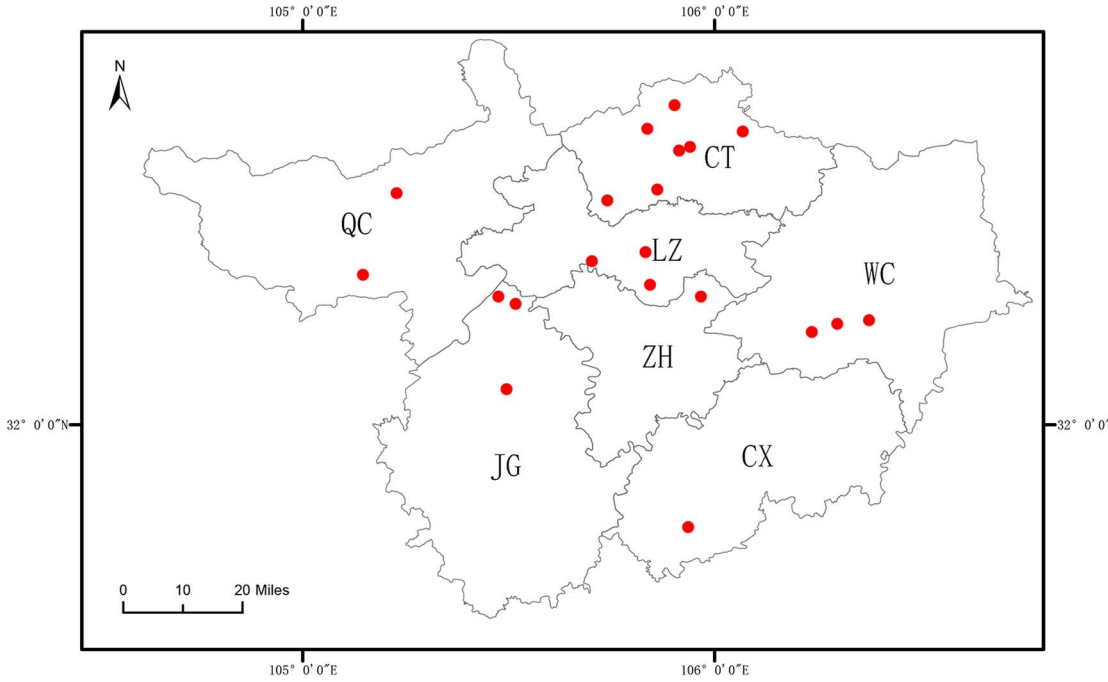

Fig. 1. Geographical position of the sampling sites. CT = Chao-Tian District; CX = Cang-Xi County; JG = Jian-Ge County; LZ = Li-Zhou District; QC = Qing-Chuan County, WC = Wang-Cang County; ZH = Zhao-Hua District. The red dots stand for the sampling sites.

and 0.5% malathion (organophosphate), used for bioassays. These insecticide-impregnated papers were made based on discriminating doses established on a laboratory susceptible strain of *Ae. albopictus* (Wang et al. 2017) and provided by the Chinese Center for Disease Control and Prevention. Four to 5 replicates of 20 to 25 3- to 5-day old, non-blood-fed female *Ae. albopictus* were exposed to the insecticide impregnated papers for 1 h and then transferred to the holding tubes with 10% sugar solution. The mortality rate was recorded 24 h later. Three statuses of insecticide susceptibility (susceptible, possible resistance, and resistance) were defined by mortality rates of between 98% and 100%, between 90% and 98%, and less than 90%, respectively.

Extraction of genomic DNA

Genomic DNA (gDNA) was extracted by the method of Rinkevich et al. (2006) with minor modifications. Briefly, individual adults were homogenized in a tube with 0.4 ml of lysis buffer (100 mM Tris-Cl pH 8.0, 50 mM NaCl, 10 mM EDTA, and 1% (w/v) SDS), followed by the addition of 3 µl (0.4 U/µl) of proteinase K and incubated at 60°C for 2 h. The samples were mixed after the addition of 60 µl of 8 M potassium acetate, and then placed on ice for 10 min. After spinning at 14,000 × g for 10 min, 320 µl of supernatant was transferred to a new tube. Then, 640 µl of ice-cold absolute ethanol was added and the samples were kept at room temperature for 20 min. The samples were spun at 14,000 × g for 10 min. Pellets were washed in 0.2 ml of 75% ethanol and spun at 14,000 × g for 10 min. The pellet was dried and then re-suspended in 25 µl of ddH_2O. The DNA samples were preserved at −20°C until use.

Amplification of two *VGSC* fragments

The primers V2F (5'- GAC AAT GTG GAT CGC TTC CC -3') and V2R (5'- GCA ATC TGG CTT GTT AAC TTG -3') (Kasai et al. 2011) were used to amplify a DNA fragment covering codons 1016 (Domain II). Primer pair V3F (5'- GAG AAC TCG CCG ATG AAC-3') and V3R (5'- TAG CTT TCA GCG GCT TCT TC -3') (Kasai et al. 2011) were used to amplify a fragment containing codons 1532 and 1534 (Domain III). Polymerase chain reaction (PCR) was performed in a final volume of 20 µl, comprising 10 µl of 2xEx Taq Master Mix (Takara Bio, Dalian, China), 1µl of DNA template and 10 µM of each primer. The reaction procedure was as follows: 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 2 min. The PCR products were gel (1%) purified and directly Sanger sequenced using primer V2F or V3R (TSINGKE, Beijing).

DNA sequence analysis and haplotype identification

The DNA sequences covering the regions that encode Domain II (D2) and Domain III (D3) of the

Downloaded from http://meridian.allenpress.com/jamca/article-pdf/40/1/20/3336206/i1943-6270-40-1-20.pdf by guest on 30 May 2024

Table 1. Mortality rates of *Aedes albopictus* adults originated from 7 districts[1] after exposure to four insecticides for 24 h.

Insecticides	Population	n	24h mortality (%)	Resistance status
0.4% Beta cypermethrin	LZ	133	96.99	possible resistance
	CT	126	98.41	susceptible
	ZH	103	90.29	possible resistance
	CX	122	93.44	possible resistance
	JG	127	93.7	possible resistance
	QC	109	96.33	possible resistance
	WC	120	93.33	possible resistance
0.1% deltamethrin	LZ	127	94.49	possible resistance
	CT	102	90.2	possible resistance
	ZH	100	88	resistance
	CX	127	94.49	possible resistance
	JG	120	96.67	possible resistance
	QC	117	94.87	possible resistance
	WC	109	83.49	resistance
0.05% propoxur	LZ	126	99.21	susceptible
	CT	104	97.12	possible resistance
	ZH	115	97.39	possible resistance
	CX	123	98.37	susceptible
	JG	104	98.08	susceptible
	QC	101	99.01	susceptible
	WC	109	97.25	possible resistance
0.5% malathion	LZ	126	100	susceptible
	CT	111	100	susceptible
	ZH	107	100	susceptible
	CX	100	100	susceptible
	JG	106	100	susceptible
	QC	113	100	susceptible
	WC	112	99.11	susceptible

[1] CT = Chao-Tian District; CX = Cang-Xi County; JG = Jian-Ge County; LZ = Li-Zhou District; QC = Qing-Chuan County; WC = Wang-Cang County; ZH = Zhao-Hua District.

VGSC were aligned by MUSCLE v.3.8 (Edgar 2004). The nucleotide polymorphisms (SNPs) in these DNA sequences were documented. The haplotypes of VGSC alleles were identified by directly reading from homozygotes, or by phasing one from the other from heterozygotes carrying one-site variations, or by clone sequencing of heterozygotes with multiple-site variations. For clone sequencing, purified PCR products were ligated with the pClone 007 (TSINGKE, Beijing) and transformed into competent cells of the *Escherichia coli* Trans 5α strain (TSINGKE, Beijing). Then, 4 to 8 positive clones were sequenced to clarify haplotypes. The haplotype sequences (GenBank accession numbers for these sequences are OR880648–OR880670) were used for phylogenetic analysis by the maximum likelihood method using MEGA 7 (Kumar et al. 2016).

RESULTS

Insecticide susceptibility

The mortality rates post-exposure to 4 insecticides for 24 h are presented in Table 1. All the populations showed possible resistance to the pyrethroid beta-cypermethrin with the exception of the CT population. For deltamethrin, suspected resistance or significant resistance (WC and ZH) was detected. Four populations were susceptible to propoxur, whereas the other 3

Table 2. Distribution and frequency of VGSC genotypes at codons 1016, 1532 and 1534 in *Aedes albopictus* collected from 7 districts of Guangyuan City, Sichuan Province, China.

		1016			1532			1534					
Location	n	V/V	V/G	G/G	I/I	I/T	T/T	F/F	F/S	S/S	F/L	L/L	L/S
CT	36	80.6	19.4	0	94.4	5.6	0	91.7	8.3	0	0	0	0
CX	35	91.4	8.6	0	94.3	5.7	0	2.9	8.6	68.6	2.9	5.7	11.4
JG	36	83.3	16.7	0	94.4	2.8	2.8	47.2	41.7	11.1	0	0	0
LZ	40	80	17.5	2.5	85	15	0	72.5	22.5	5	0	0	0
QC	36	97.2	2.8	0	94.4	5.6	0	69.4	22.2	8.3	0	0	0
WC	36	88.9	11.1	0	91.7	8.3	0	11.1	13.9	75	0	0	0
ZH	36	69.4	27.8	2.8	86.1	13.9	0	61.1	33.3	5.6	0	0	0

Downloaded from http://meridian.allenpress.com/jamca/article-pdf/40/1/20/3336206/i1943-6270-40-1-20.pdf by guest on 30 May 2024

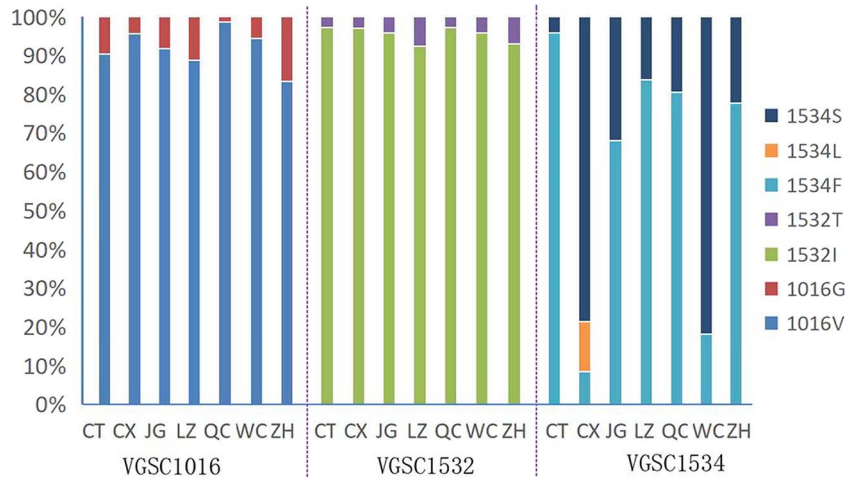

Fig. 2. Distribution and frequencies of *kdr* alleles in 7 *Aedes albopictus* populations.

populations (CT, WC, and ZH) showed possible resistance with mortality rates of approximately 97%. All populations were susceptible to malathion.

Distribution and frequency of *kdr* mutations in Guangyuan *Aedes albopictus*

The V1016G (GTA to GGA) mutation in D2 of the VGSC was detected in this study. The 1016G heterozygotes were present in all 7 districts, whereas the 1016G homozygote was detected only in LZ and ZH at a frequency below 5% (Table 2). The frequencies of the resistance allele 1016G ranged from 1.4% (QC) to 16.7% (ZH) (Fig. 2). A non-synonymous variation at codon 1532 (ATC to ACC), resulting in the amino acid substitution I to T, was also identified. Except in JG, where one 1532T homozygote was detected, 1532T was observed in the heterozygous form in all of the 7 populations (Table 2). In addition, two variations were observed at codon 1534 leading to two resistant mutations F1534L (TTC to TTG) and F1534S (TTC to TCC). The mutation F1534L was only found in CX with a frequency of 12.9%, whereas F1534S was commonly present with frequencies ranging from 4.2% (CT) to 81.9% (WC) (Table 2, Fig. 2). Notably, the frequencies of 1534S homozygote were high in CX (68.6%) and WC (75%) (Table 2).

Combining the 3 *kdr*-related sites of *VGSC* (1016, 1532, 1534) together, 13 types of triple-site genotype combinations were documented in a total of 255 individuals (Table 3). Notably, the triple-site wild homozygote (Type 1) was not present in CX but had a high frequency in CT. Individuals heterozygous for 1534S (Type 8) were commonly distributed in Guangyuan at frequencies ranging from 2.8% (WC) to 33.3% (JG). Single-site mutant homozygotes (Types 2–5) or double-site mutant heterozygotes (Types 10-13) were present, whereas there were no double-site mutant homozygotes or triple-site mutant individuals observed.

Table 3. Distribution and frequency of *VGSC* triple-site genotype combinations in *Aedes albopictus* collected from 7 districts of Guangyuan City, Sichuan Province, China.

	sites			Population						
Type	1016	1532	1534	CT	CX	JG	LZ	QC	WC	ZH
Type 1	V/V	I/I	F/F	72.2	0	33.3	47.5	61.1	5.6	27.8
Type 2	**G/G**	I/I	F/F	0	0	0	2.5	0	0	2.8
Type 3	V/V	**T/T**	F/F	0	0	2.8	0	0	0	0
Type 4	V/V	I/I	**S/S**	0	68.6	11.1	5	8.3	75	5.6
Type 5	V/V	I/I	**L/L**	0	5.7	0	0	0	0	0
Type 6	V/**G**	I/I	F/F	13.9	0	11.1	12.5	2.8	2.8	16.7
Type 7	V/V	**I/T**	F/F	0	0	0	7.5	5.6	0	8.3
Type 8	V/V	I/I	F/**S**	8.3	2.9	33.3	15	22.2	2.8	27.8
Type 9	V/V	I/I	**L/S**	0	11.4	0	0	0	0	0
Type 10	V/**G**	I/I	F/**L**	0	2.9	0	0	0	0	0
Type 11	V/**G**	I/I	F/**S**	0	2.9	5.6	2.5	0	5.6	5.6
Type 12	V/V	**I/T**	F/**S**	0	2.9	2.8	5	0	5.6	0
Type 13	V/**G**	**I/T**	F/F	5.6	2.9	0	2.5	0	2.8	5.6

Downloaded from http://meridian.allenpress.com/jamca/article-pdf/40/1/20/3336206/i1943-6270-40-1-20.pdf by guest on 30 May 2024

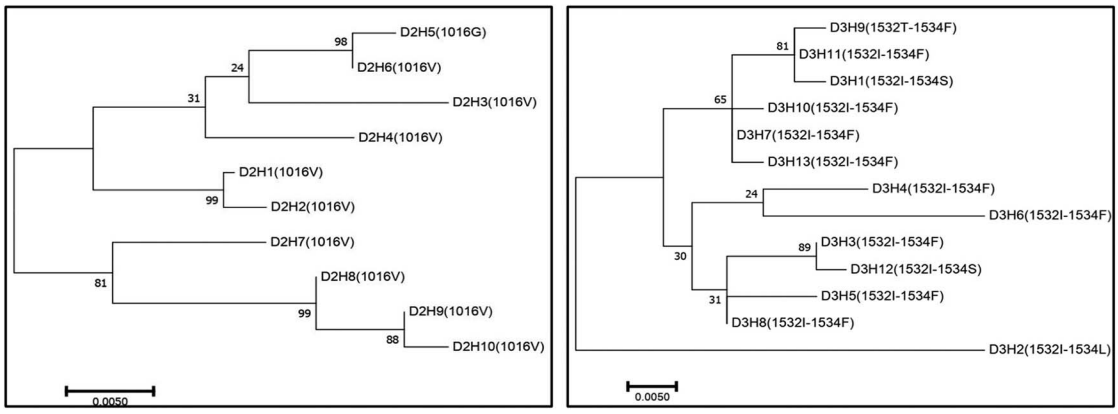

Fig. 3. Molecular phylogenetic analysis by the maximum likelihood method. The evolutionary history was inferred based on the Tamura-Nei model (Tamura and Nei 1993). The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. GenBank accession numbers for D2 sequences are OR880648–OR880657, for D3 are OR880658–OR880670.

Identification of *VGSC* haplotypes in Guangyuan *Aedes albopictus*

From our samples, 10 and 13 haplotypes based on the DNA sequences of D2 and D3, respectively, were identified. Among these, 1 1016G (D2H5), 1 1532T (D3H9), 1 1534L (D3H2) and 2 1534S resistant haplotypes (D3H1 and D3H12) were identified in our samples. The phylogenetic relationships of these haplotypes in the maximum likelihood trees showed that the resistant 1016G haplotype (D2H5) was closely clustered with the wild D2H06 in one of the 2 major clades, and the 2 haplotypes with a 1534S mutation (D3H1 and D3H12) were located in different clades (Fig. 3).

DISCUSSION

The bioassay data indicated that none of the 7 populations were highly resistant to the 4 recommended insecticides. However, possible resistance to propoxur, beta cypermethrin, and deltamethrin was detected. Notably, 2 populations exhibited significant resistance to deltamethrin. All populations were susceptible to malathion. Based on these observations, it is suggested to reduce the use of deltamethrin in Guangyuan City, especially in WC and ZH.

The previously reported V1016G mutations in D2, and I1532T, F1534L, and F1534S in D3 of the *VGSC* (Kasai et al. 2019, Zhou et al. 2019) were detected, whereas the F1534C mutation (Gao et al. 2018, Zhou et al. 2019) was not observed in this study. Overall, although varying frequencies were detected in different locations, the resistant alleles 1016G, 1532T, and 1534S were widespread in Guangyuan City. It has been characterized that mutations 1016G and 1534S could confer resistance to pyrethroids (Kasai et al. 2019), and the existence of these two mutations would predict a risk of pyrethroid resistance in Guangyuan. Actually, in WC and ZH, where the highest frequencies of 1534S (81.9% in WC) and 1016G (16.7% in ZH) were detected, phenotypic resistance to deltamethrin was observed (Table 1 and Fig. 2). These data suggest that more attention should be paid to the dynamics of insecticide resistance in these 2 districts.

The DNA sequence analysis showed that the resistant 1016G haplotype identified in this study (D2H5) was identical to that reported in Beijing, China (MK201608, Zhou et al. 2019), and Italy (MW375107 and MW375109) (Pichler et al. 2021), and the haplotype carrying the 1532T mutation (D3H9) was the same in sequence as that observed in Beijing (Zhou et al. 2019) and Shanghai (Gao et al. 2018). There was not an exact identical sequence with the 1534L haplotype (D3H2), but several highly similar sequences to the 1534L haplotype were released in GenBank. The two haplotypes (D3H1 and D3H12) that harbor the 1534S mutation were also reported in samples from Beijing (Zhou et al. 2019). The data obtained in this study (Fig. 3) support our previous notion that the 1016G mutation has a single evolutionary origin, whereas 1534S may occur on different genetic backgrounds in *Ae. albopictus* found in China (Zhou et al. 2019).

In conclusion, our data revealed the occurrence of phenotypic resistance and multiple-site *kdr* mutations in *Ae. albopictus* from the 7 locations of Guangyuan City. Therefore, regular monitoring of phenotypic resistance is highly recommended to make the best decisions when using insecticides for mosquito control in this city.

REFERENCES CITED

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797. http://dx.doi.org/10.1093/nar/gkh340

Gao J, Chen H, Shi H, Peng H, Ma Y. 2018. Correlation between adult pyrethroid resistance and knockdown

Downloaded from http://meridian.allenpress.com/jamca/article-pdf/40/1/20/3336206/i1943-6270-40-1-20.pdf by guest on 30 May 2024

resistance (kdr) mutations in *Aedes albopictus* (Diptera: Culicidae) field populations in China. *Infectious Dis Poverty* 7:1–9. https://doi.org/10.1186/s40249-018-0471-y

Gratz NG. 2004. Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol* 18:215–227. https://doi.org/10.1111/j.0269-283X.2004.00513.x

Jia Y, Yang Y, Luo X, Zhao J. 2018. Monitoring and analysis of drug resistance of *Aedes albopictus* in Guangyuan in 2015. *Parasites Infect Dis* 16:41–43. (In Chinese)

Kasai S, Caputo B, Tsunoda T, Cuong TC, Maekawa Y, Lam-Phua S G, Pichler V, Itokawa K, Murota K, Komagata O, Yoshida C, Chung HH, Bellini R, Tsuda Y, Teng HJ, de Lima JL, Alves LC, Ng LC, Minakawa N, Yen NT, Phong TV, Sawabe K, Tomita T. 2019. First detection of a *vssc* allele V1016G conferring a high level of insecticide resistance in *Aedes albopictus* collected from Europe (Italy) and Asia (Vietnam), 2016: a new emerging threat to controlling arboviral diseases. *Euro Surveill* 24:1700847. https://doi.org/10.2807/1560-7917.ES.2019.24.5.1700847

Kasai S, Ng LC, Lam-Phua SG, Tang CS, Itokawa K, Komagata O, Kobayashi M, Tomita T. 2011. First detection of a putative knockdown resistance gene in major mosquito vector, *Aedes albopictus*. *Jpn J Infect Dis* 64:217–221.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Li Y, Xu J, Zhong D, Zhang H, Yang W, Zhou G, Su X, Wu Y, Wu K, Cai S, Yan G, Chen X. 2018. Evidence for multiple-insecticide resistance in urban *Aedes albopictus* populations in southern China. *Parasit Vectors*. 11:1–10. https://doi.org/10.1186/s13071-017-2581-y

Liu B, Gao Z, Ma J, Jia Z, Xiao J, Haat MA, Wang H. 2019. Modeling the present and future distribution of arbovirus vectors *Aedes aegypti* and *Aedes albopictus* under climate change scenarios in Mainland China. *Sci Total Environ* 664 203–214. https://doi.org/10.1016/jscitotenv.2019.01.301

Luo X, Liu Y, Jia Y. 2021. Bionomic characteristics of dengue vector *Aedes mosquitoes* in Guangyuan of Sichuan province, China, 2018-2020. *Chin J Vector Biol Control* 32:705–709. https://doi.org/10.11853/j.issn.1003.8280.2021.06.010

Marcombe S, Farajollahi A, Healy SP, Clark GG, Fonseca DM. 2014. Insecticide resistance status of United States populations of *Aedes albopictus* and mechanisms involved. *PLoS One* 91–10. https://doi.org/10.1371/journal.pone.0101992

Moyes CL, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, Raghavendra K, Pinto J, Corbel V, David JP, Weetman D. 2017. Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Negl Trop Dis* 11:1–20. https://doi.org/10.1371/journal.pntd.0005625

Pichler V, Mancini E, Micocci M, Calzetta M, Arnoldi D, Rizzoli A, Lencioni V, Paoli F, Bellini R, Veronesi R, Martini S, Drago, A, De Liberato C, Ermenegildi A, Pinto J, della Torre A, Caputo B. 2021. A novel allele specific polymerase chain reaction (AS-PCR) assay to detect the V1016G knockdown resistance mutation confirms its widespread presence in *Aedes albopictus* populations from Italy. *Insects* 12:79. https://doi.org/10.3390/insects12010079

Rinkevich FD, Zhang L, Hamm RL, Brady SG, Lazzaro BP, Scott JG. 2006. Frequencies of the pyrethroid resistance alleles of *Vsscl* and *CYP6D1* in house flies from the eastern United States. *Insect Mol Biol* 15:157–167. https://doi.org/10.1111/j.1365-2583.2006.00620.x

Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 10:512–526.

Wang Y, Liu X, Li C, Su T, Jin J, Guo Y, Ren D,Yang Z, Liu Q, Meng F. 2017. A survey of insecticide resistance in *Aedes albopictus* (Diptera: Culicidae) during a 2014 Dengue fever outbreak in Guangzhou, China. *J Econ Entomol* 110:239–244.

WHO [World Health Organization]. 2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd edition. Geneva: World Health Organization.

Xu J, Bonizzoni M, Zhong D, Zhou G, Cai S, Li Y, Wang X, Lo E, Lee R, Sheen R, Duan J, Yan G, Chen XG 2016. Multi-country survey revealed prevalent and novel F1534S mutation in voltage-gated sodium channel (*VGSC*) gene in *Aedes albopictus*. *PLoS Negl Trop Dis* 10:1–12. https://doi.org/10.1371/journal.pntd.0004696

Zhou X, Yang C, Liu N, Li M, Tong Y, Zeng X, Qiu X. 2019. Knockdown resistance (*kdr*) mutations within seventeen field populations of *Aedes albopictus* from Beijing China: first report of a novel V1016G mutation and evolutionary origins of *kdr* haplotypes. *Parasit Vectors* 12:1–16. https://doi.org/10.1186/s13071-019-3423-x

Downloaded from http://meridian.allenpress.com/jamca/article-pdf/40/1/20/3336206/i1943-6270-40-1-20.pdf by guest on 30 May 2024