Journal of Medical Entomology, XX(X), 2018, 1–5
doi: 10.1093/jme/tjy121
Short communication

OXFORD

Vector Control, Pest Management, Resistance, Repellents

Frequency of the L1014F Mutation in the Sodium Channel Gene, in *Culex quinquefasciatus* (Diptera: Culicidae) Populations From Rural and Urban Areas of Yucatan State, Mexico

Wilbert A. Chi-Chim,[1] Virgilio Bocanegra-Garcia,[1] Guadalupe Reyes-Solis,[2] Julian E. García-Rejon,[2] Carlos M. Baak-Baak,[2] Carlos Machain-Williams,[2] Julio A. Chan-Orilla,[2] Consuelo Gomez-Garcia,[3] Horacio S. Ballina-Gomez,[4] and Miguel Angel Reyes-Lopez[1,5]

[1]Conservation Medicine Lab. Centro de Biotecnologia Genomica, Instituto Politecnico Nacional, Blvd. del Maestro S/N esq. Elias Piña, Narcizo Mendoza, Cd. Reynosa, Tam. 88740, Mexico, [2]Laboratorio de Arbovirologia, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autonoma de Yucatan, calle 43 N. 613 x calle 90 colonia Inalambrica, Merida, Yucatan, Mexico, [3]Laboratorio de Biomedicina Molecular I. Escuela Nacional de Medicina y Homeopatía del Instituto Politécnico Nacional (ENMyH-IPN), México, [4]Instituto Tecnologico de Conkal, Km 16.3 Antigua carretera a Merida-Motul, Conkal, Yucatan, C.P. 97345, and [5]Corresponding author, e-mail: mareyesl@gmail.com

Subject Editor: David Severson

Received 7 March 2018; Editorial decision 6 July 2018

Abstract

Culex quinquefasciatus Say (Diptera: Culicidae) is a mosquito species that has attracted a lot of attention from a medical and veterinary point of view; however, little is known about the frequency of L1014F mutations that have been found in the sodium channel gene, with this being a target for DDT and pyrethroid insecticides. The distribution and frequency of the L1014F mutation in *Cx. quinquefasciatus* populations was determined in rural and urban areas of Yucatan, Mexico from January 2015 to March 2016. Nine hundred fifty adult females out of 17,727 immature states were collected and analyzed in all sites sampled ($n = 10$). Susceptible homozygotes were identified (L1014/L1014) in 12% (114/950), heterozygous individuals (F1014/L1014) in 34% (323/950), and mutated homozygotes (F1014/F1014) in 54% (513/950) during the dry and rainy seasons. In this work, study areas with a high frequency of L1014F mutation were identified. These findings may help guarantee a more effective and efficient use of the resources available for the control of this vector.

Key words: *Culex quinquefasciatus*, L1014F-*kdr*, frequency, rural and urban areas, Yucatan

Culex quinquefasciatus Say (Diptera: Culicidae) is a member of the *Culex pipiens* complex with a worldwide distribution and of medical and veterinary importance. It is one of the main vectors of *Wuchereria bancrofti* that causes lymphatic filariasis (White 1989), West Nile Virus (WNV), Rift Valley fever virus, Saint Louis encephalitis virus, and Sindbis virus under laboratory conditions (Turell 2012). It is currently not clearly known to be a Zika virus vector (Hart et al. 2017). However, in Brazil, it was reported that a vector was infected with this virus (Duschinka et al. 2017), Mexico (D Elizondo-Quiroga et al. 2018). Additionally, WNV was detected in *Cx. quinquefasciatus* in Nuevo Leon, Mexico (Elizondo-Quiroga et al. 2005). In Yucatan, Mexico, several bird and mammal species have been seropositive for WNV by the plaque reduction neutralization test (Farfan-Ale et al. 2006). *Cx. quinquefasciatus* is distributed throughout the state of Yucatan, Mexico (Baak -Baak et al. 2017); therefore, this state would be considered a high-risk area for the transmission of arboviruses by this vector. The general control of disease-transmitting mosquito vectors is based on the application of insecticides, such as pyrethroid insecticides, which are mostly used for indoor residual spraying and mosquito net impregnation due to their low toxicity to mammals and their rapid knock-down effect on insects. However, these control strategies applied to *Aedes aegypti* are probably indirectly affecting the *Cx. quinquefasciatus* populations. (Ndiath et al. 2012, Ponce et al. 2016). In Mexico, a kdr Ile1,016 mutation was reported in populations of *Ae. aegypti* and was associated with resistance to pyrethroid insecticides (Deming et al. 2016, Saavedra Rodriguez et al.2007); this indicates significant differences in the frequency of mutations associated with resistance. Insect species have presented mutations that confer resistance to knock down and reduce the binding of insecticides to sodium channels, which is the target of DDT insecticides and pyrethroids (Williamson et al. 1996, Dong 1997, Martinez-Torres et al.1998, 1999, Chen et al. 2017). Studies conducted (Ponce et al. 2016) in *Cx. quinquefasciatus* populations from the states of Nuevo Leon and Coahuila, which are located in northeastern Mexico, have found an L1014F mutation, which is a substitution of leucine by phenylalanine at position 1014 that causes sodium channel sensitivity (O'Reilly et al. 2006, Wondji et al. 2008). However, little is known about the frequency of the L1014F mutation in *Cx. quinquefasciatus* mosquito populations in southeastern Mexico, a broad area with conditions conducive to proliferation of these vectors. Thus, we considered it necessary to conduct studies to determine the current frequency of the L1014F mutation in different areas of the state of Yucatan, Mexico to reinforce health and sanitary measures that are applied in this state.

Materials and Methods

Study Area

This study was carried out in 10 localities of the state of Yucatan, located on the northern tip of the Yucatan Peninsula in southeastern Mexico, bordered on the north by the Gulf of Mexico, on the east and southeast by Quintana Roo, and on the west and southwest by Campeche. The 10 areas were selected for this study, based on frequent reports of dengue cases obtained in studies performed with the dengue vector *Ae. aegypti* in areas with a high rate of dengue cases including urban areas with >10,000 inhabitants and rural areas with <10,000 (www.censo2010.org.mx/; last accessed December 2014).

Collection Sites

Approximately 17,727 immature states of *Cx. quinquefasciatus* were collected in 508 artificial breeding sites in January 2015 and March 2016 from 10 sites of the state of Yucatan, Mexico during the rainy and dry seasons. The larvae were transported to the insectarium of the Arbovirology laboratory to be identified until reaching the adult stage; fifty females were selected for each season, with a total of 100 females for each site.

Genomic DNA Extraction

We selected fifty 2-d-old female mosquitoes from each locality according to WHO criteria (1998). The mosquitoes were placed individually in 1.5-ml Eppendorf tubes for maceration and extraction of genomic DNA with the salt technique according to Black and DuTeau (1997). The concentration and purity of each sample were measured in a NanoDrop 2000 (Thermo Fisher Scientific). Then, we adjusted final concentration to 50 ng/µl for each of the samples.

Molecular Tests to Determine the Presence of 1014F Mutation

PCR was carried out; the first reaction was to amplify a fragment of the sodium channel gene of *Cx. quinquefasciatus*, the second reaction was to determine the presence of the L1014F (substitution of leucine to phenylalanine) mutation and susceptible alleles using specific primers according to Martínez-Torres et al. (1999) and Xu et al. (2011). The following temperature conditions were used in the first reaction, 95°C for 5 min, followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min with one step of final extension at 72°C for 10 min. The conditions for the second reaction were 95°C for 1 min, 38 cycles of 94°C for 30 s, and 72°C for 1 min, 72°C for 10 min.

Data Analysis

The genotypes were calculated using a previous equation reported (Garcia et al. 2009), dividing the number of individuals with a given genotype by the total number of mosquitoes analyzed as follows: 1) homozygous susceptible genotype frequency L1014/L1014, 2) homozygous mutant genotype frequency, F1014/F1014, and 3) heterozygous genotype frequency, L1014/F1014.

Results

Presence and Frequency of the 1014F Mutation

In total 950 *Cx. quinquefasciatus* individuals were analyzed in the two seasons. In the dry season, samples were not collected in the municipality of Motul, the frequency of homozygous susceptible (L1014/L1014) was 12% (114/950), heterozygotes (F1014/ L1014) 34% (323/950), and homozygous mutated (F1014/F1014) 54% (513/950). When grouped by season, of the 450 females analyzed during the dry season, 14% (63/450) was identified as homozygous susceptible, 31.33% (141/450) as heterozygotes, and 54.67% (246/450) as homozygous mutated. In the rainy season, 500 females were analyzed with 10.2% (51/500) identified as homozygous susceptible, 36.4% (182/500) as heterozygotes, and 53.4% (267/500) as homozygous mutated. In the dry season, the presence of homozygous-mutated individuals, F1014/F1014, was similar in urban and rural areas with 58.4 and 50%, respectively (Fig. 1). However, differences were observed by study site. In the urban areas of Juan Pablo, Uman, and Kanasin, the L1014F mutation in *Cx. quinquefasciatus* was high, ranging from 71 to 98%. In the rural areas, the L1014F mutation was 96% in *Cx. quinquefasciatus* collected in Xkalakdzonot, 62% in Caucel, and 64% in Chochola (Table 1).

In the rainy season, urban areas, such as Juan Pablo II and Kanasin, maintained the F1014 mutation. A decreased frequency of the F1014 mutation in *Cx. quinquefasciatus* from Uman was observed, but the mutant gene was more frequent in mosquitos of Col. Centro. Notably, the frequency of the L1014F mutation was high in *Cx. quinquefasciatus* from all rural areas studied, ranging from 63 to 87% (Supplementary Table S2).

Discussion

High L1014F mutation frequencies have been determined in *Cx. quinquefasciatus* populations in 10 important sites in the state of Yucatan, Mexico, where strategies have been applied for the control of *Ae. aegypti*, a vector that has developed resistance to pyrethroid insecticides due to the frequency of mutations (Ile1016) (Flores et al. 2006, Garcia-Rejon et al. 2018) found in the sodium channel gene. It has been shown that resistance to insecticides is not only caused by mutations in the sodium channel gene, but also by enzymatic mechanisms involved in their development (Brengues et al. 2003, Rodriguez et al. 2005, Deming et al. 2016). However, studies based on resistance in populations of *Ae. aegypti* in Merida City, Yucatan, have presented different variations in mutation frequencies associated with resistance to groups of insecticides such as chlorpyrifos (Deming et al. 2016) and organophosphate (Flores et al. 2006, Aponte et al. 2013), in places near the city of Merida (Flores et al. 2012). In the *Cx. quinquefasciatus* populations in Yucatan, Mexico, the frequency of the L1014F mutation related to insecticide resistance has been similar to studies conducted by Ponce et al. (2016) in northeastern Mexico, where the presence of the mutation was determined in 16 *Cx. quinquefasciatus* populations; nevertheless, the presence of this mutation has also been reported in *Anopheles* spp.,

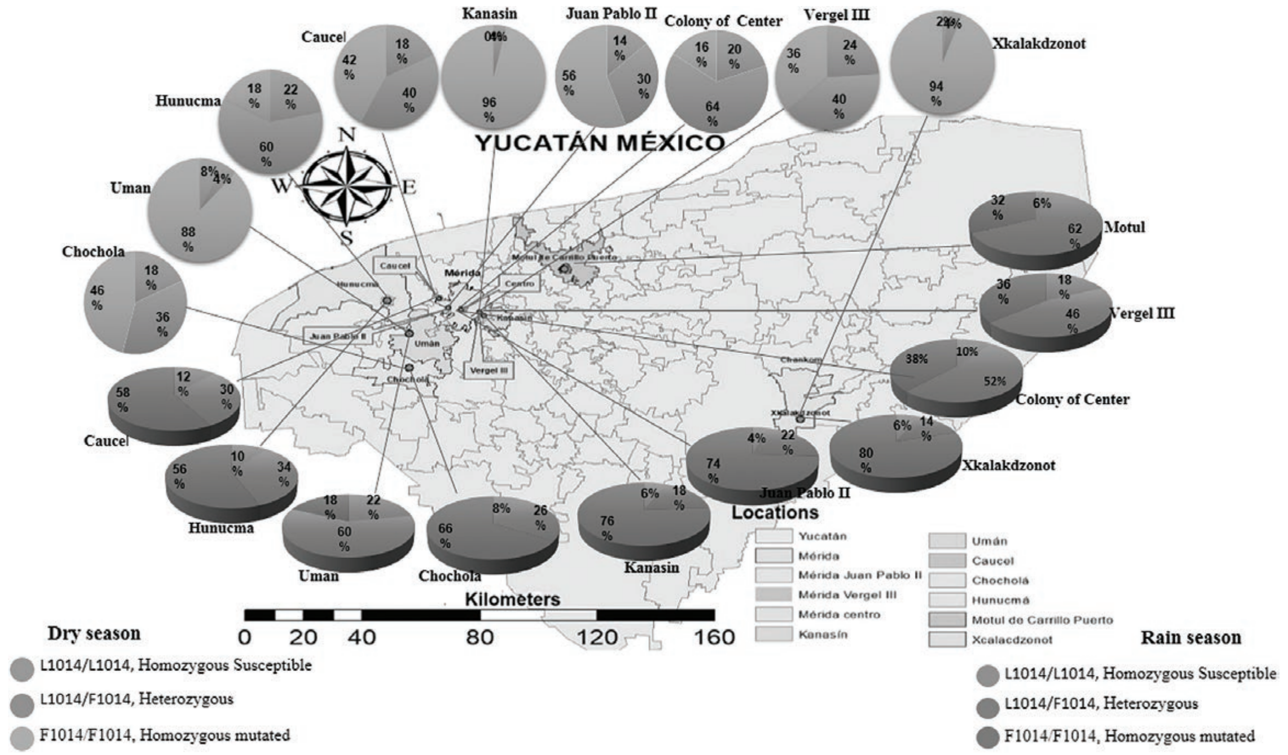

Fig. 1. Distribution of alleles L1014/L1014, L1014/F1014 and F1014/F1014 in populations of *Cx. quinquefasciatus* during the dry (from Chocola to Xkalakdzonot, upper part) and rain (from Causel Motul, lower part) seasons. Circular plots represent the percentage of alleles present at each site.

Table 1. Genotypes present in *Cx. quinquefasciatus* mosquito populations during the dry season in 2015

							Allele frequency	
Zone	Colony/Municipality	Year	No. of mosquitoes	AA	AT	TT	R	S
Urban	Col. Juan Pablo II	2015	50	7	15	28	0.71	0.29
	Col. Vergel III	2015	50	12	20	18	0.56	0.44
	Col. Centro	2015	50	10	32	8	0.48	0.52
	Kanasin	2016	50	0	2	48	0.98	0.02
	Uman	2016	50	4	2	44	0.90	0.10
	Hunucma	2015	50	11	30	9	0.48	0.52
	Motul	2015	50	–	–	–	–	–
Rural	Caucel	2015	50	9	20	21	0.62	0.38
	Chochola	2015	50	9	18	23	0.64	0.36
	Xkalaktzonot	2016	50	1	2	47	0.96	0.04

Col. = colony; AA = homozygous Susceptible; AT = heterozygous; TT = homozygous mutated.

three *Culex* spp., and *Cx. pipiens* (Qiang et al. 2011, Dong et al. 2014, Scott et al. 2015, Bkhache et al. 2016) in urban and rural sites in Morocco, modifying the structure of the sodium channel (Singh et al. 2015, Bkhache et al. 2016, Niang et al. 2016). The control programs focused on *Ae. aegytpi* have had an indirect impact on *Cx. quinquefasciatus* populations (Flores et al. 2013, Ponce et al. 2016, Vazquez-Prokopec et al. 2017).

The frequencies of the L1014F mutations observed in the *Cx. quinquefasciatus* populations in rural and urban areas sampled in our study probably may be related to their feeding habitat, their reproduction in wastewater as well as in containers contaminated with insecticides that are used by farmers and which cause changes in the epidermal structure of the insect. This makes them more tolerant to insecticides and increases the production of metabolic enzymes and mutations that affect the target sites of insecticides (Osta et al. 2012).

The distribution of the frequency of the L1014F mutation, four sites in the urban area (Col. Juan Pablo II, Col. Centro, Uman, and Kanasin), revealed high frequencies of the mutated allele (1014F) during the dry and rainy seasons, whereas in the rural area, the municipality of Xkalaktzonot was the site that showed high frequencies of the mutated allele (1014F) in the two seasons, showing a statistically significant difference in the frequency of the mutation in *Cx. quinquefasciatus* populations in the dry season ($X^2 = 108.48$, $P \leq 0.05$) and the rainy season ($X^2 = 161.78$, $P \leq 0.05$). Also shown that there is an association between the mutation frequency and the *Cx. quinquefasciatus* collection area in rural communities ($X^2 = 10.413$, $gl = 4$, $P = 0.034$) and urban areas ($X^2 = 19.257$, $gl = 4$, $P = 0.001$). These findings are probably due to the use of personal consumption products, such as household insecticides as mentioned by Loroño-Pino et al. (2014) as well as the frequent use of pyrethroids for the control of *Ae. aegypti* around houses, generating a selection pressure in urban areas mentioned by Saavedra-Rodriguez et al. (2015). This causes the increase of mutant 1014F alleles related to insecticide resistance, an event that could explain the high frequencies found in some urban areas. On the other hand, rural areas such as Motul and Xkalakdzonot showed high percentages of 1014F mutations in mosquito populations collected in these sites. It is worth mentioning that the municipality of Xkalakdzonot was the one that presented the highest frequency of mutant 1014F alleles in both seasons of the year (rainy and dry). This shows high values with respect to other sites in the rural area. High frequency of the L1014F mutation is probably due to several factors, such as the gene flow in a range of 180 km (Gorrochotegui 2002, Sarkar et al. 2009), as well as the exposure of these vectors to the insecticides used in agriculture (Molly 2016). In many rural communities, people work in crop fields with various types of insecticides, herbicides, and fungicides available on the market and apply them for the control of pests. This increases the probability of inducing resistance to insecticides (Nkya et al. 2014, Molly 2016). This possibly explains the differences observed in each site.

In this work, we conclude that the L1014F mutation related to insecticide resistance in *Cx. quiquefasciatus* populations in urban and rural areas in the state of Yucatan, Mexico is present in all sites and at different times of the year and that there are variants of allelic frequency depending on the weather conditions of the year, which is of great importance for early detection to exercise control by selecting suitable insecticides.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

We are grateful for the support of the Centro de Biotecnologia Genomica of the Instituto Politecnico Nacional and the Laboratory of Arbovirology of the Universidad Autonoma de Yucatan for the collection of biological material as well as for the use of their insectarium equipment. The study was supported in part by the Consejo Nacional de Ciencia y Tecnología (CONACyT), Mexico, Fondos Mixtos CONACYT (Project Nº 180879), and the Secretaria de Investigacion y Posgrado, Instituto Politecnico Nacional; Grants: 20161179, 20171851. W. C. C. was a recipient of a fellowship from CONACyT, Mexico. M.A.R.L., V.B.G., and C.G.G. are SNI (National Researchers System in Mexico) members and fellows of COFAA and EDI from Instituto Politecnico Nacional. Project: Development and implementation of participatory actions for the prevention of environmental impacts of solid waste management in rural communities of Yucatan (CIRB-2016-0013), financed by W.K. Kellogg. We also thank Jose Fernando Chan Canul and Rosa Carmina Cetina Trejo.

References Cited

Aponte, H. A., R. P. Penilla, F. Dzul-Manzanilla, A. Che Mendoza, A. D. Lopez, F. Solis, et al. 2013. The pyrethroid resistance status and mechanism in *Aedes aegypti* from the Guerrero state, Mexico. Pestic. Biochem. Physol. 107: 226–234.

Baak-Baak, C. M., D. A. Moo-Llanes, N. Cigarroa-Toledo, F. I. Puerto, C. Machain-Williams, G. Reyes-Solis, Y. J. Nakazawa, A. Ulloa-Garcia, and J. E. Garcia-Rejon. 2017. Ecological niche model for predicting distribution of disease-vector mosquitoes in yucatán state, México. J. Med. Entomol. 54: 854–861.

Black, W. C. IV, and N. M. DuTeau. 1997. RAPD-PCR and SSCP analysis for insect population genetic studies, pp. 514–531. In J. M. Crampton, C. B. Beard, C. Louis (eds.), Mol. Biol. Insect Dis. Vectors. A Methods Manual, Chapman & Hall, New York.

Bkhache, M., F. Z. Tmimi, O. Charafeddine, C. Faraj, A. B. Failloux, and M. Sarih. 2016. First report of L1014F-*kdr* mutation in *Culex pipiens* complex from Morocco. Parasit. Vectors. 9: 644.

Brengues, C., N. J. Hawkes, F. Chandre, L. McCarroll, S. Duchon, P. Guillet, S. Manguin, J. C. Morgan, and J. Hemingway. 2003. Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. Med. Vet. Entomol. 17: 87–94.

Chen, M., Y. Du, Y. Nomura, G. Zhu, B. S. Zhorov, and K. Dong. 2017. Mutations of two acidic residues at the cytoplasmic end of segment IIIS6 of an insect sodium channel have distinct effects on pyrethroid resistance. Insect Biochem. Mol. Biol. 82: 1–10.

Darwin Elizondo-Quiroga, A. M. -S., J. M. Sánchez-González, K. A. Eckert, E. Villalobos-Sánchez, A. R. Navarro-Zúñiga, G. Sánchez-Tejeda, F. Correa-Morales, C. González-Acosta, C. F. Arias, S. López, et al. 2018. Zika virus in salivary glands of five diferent species of wild-caught mosquitoes from Mexico. Sci. Rep.. 8: 809.

Deming, R., P. Manrique-Saide, A. Medina Barreiro, E. U. Cardeña, A. Che-Mendoza, B. Jones, K. Liebman, L. Vizcaino, G. Vazquez-Prokopec, and A. Lenhart. 2016. Spatial variation of insecticide resistance in the dengue vector *Aedes aegypti* presents unique vector control challenges. Parasit. Vectors. 9: 67.

Dong, K. 1997. A single amino acid change in the para sodium channel protein is associated with knockdown-resistance (*kdr*) to pyrethroid insecticides in *German cockroach*. Insect Biochem. Mol. Biol. 27: 93–100.

Dong, K., Y. Du, F. Rinkevich, Y. Nomura, P. Xu, L. Wang, K. Silver, and B. S. Zhorov. 2014. Molecular biology of insect sodium channels and pyrethroid resistance. Insect Biochem. Mol. Biol. 50: 1–17.

Duschinka, G.R., M. H. S. Paiva, M. A. Donato, P. P. Barbosa, L. Krokovsky, S. W. Dos Rocha, K. L. Saraiva, M. M. Crespo, T. M. T. Rezende, G. L. Wallau, et al. 2017. Zika virus replication in the mosquito *Culex quinquefasciatus* in Brazil. Emerg. Microbes Infect. 6: 69.

Elizondo-Quiroga, D., C. T. Davis, I. Fernandez-Salas, R. Escobar-Lopez, D. Velasco Olmos, L. C. Soto Gastalum, M. Aviles Acosta, A. Elizondo-Quiroga, J. I. Gonzalez-Rojas, J. F. Contreras Cordero, et al. 2005. West Nile Virus isolation in human and mosquitoes, Mexico. Emerg. Infect. Dis. 11: 1449–1452.

Farfán-Ale, J. A., B. J. Blitvich, N. L. Marlenee, M. A. Loroño-Pino, F. Puerto-Manzano, J. E. García-Rejón, E. P. Rosado-Paredes, L. F. Flores-Flores, A. Ortega-Salazar, J. Chávez-Medina, et al. 2006. Antibodies to West Nile virus in asymptomatic mammals, birds, and reptiles in the Yucatan Peninsula of Mexico. Am. J. Trop. Med. Hyg. 74: 908–914.

Flores, A. E., W. Albeldaño-Vázquez, I. F. Salas, M. H. Badii, H. L. Becerra, P. G. Garcia, F. S. Lozano, G. W. Brogdon, C. W. IV Black, and B. Beaty. 2005. Elevated α-esterase levels associated with permethrin tolerance in *Aedes aegypti* (L.) from Baja California, Mexico. Pestic. Biochem. Physiol. 82: 66–78.

Flores, A. E., J. S. Grajales, I. F. Salas, G. P. Garcia, M. H. Becerra, S. Lozano, W. G. Brogdon, W. C. Black, 4th, and B. Beaty. 2006. Mechanisms of insecticide resistance in field populations of *Aedes aegypti* (L.) from Quintana Roo, Southern Mexico. J. Am. Mosq. Control Assoc. 22: 672–677.

Flores, A. E., G. Ponce, M. A. Loroño, J. E. García, C. Machain, G. C. Reyes, S. Lozano, E. Lars, B. J. Beaty, and W.C. Black. 2012. Insecticide resistance in *Aedes aegypti* in Mexico: implications for dengue control (abstract). DMID Intern. Res. in Infect. Dis. Meet. Bethesda, MD. 49.

Flores, A. E., G. Ponce, B. G. Silva, S. M. Gutierrez, C. Bobadilla, B. Lopez, R. Mercado, and W. C. Black, 4th. 2013. Wide spread cross resistance to pyrethroids in *Aedes aegypti* (Diptera: Culicidae) from Veracruz state Mexico. J. Econ. Entomol. 106: 959–969.

García, G. P., A. E. Flores, I. Fernández-Salas, K. Saavedra-Rodríguez, G. Reyes-Solis, S. Lozano-Fuentes, J. Guillermo Bond, M. Casas-Martínez, J. M. Ramsey, J. García-Rejón, et al. 2009. Recent rapid rise of a permethrin knock down resistance allele in Aedes aegypti in México. Plos Negl. Trop. Dis. 3: e531.

Garcia-Rejon, J. E., J. A. Chan-Orilla, N. Cigarroa-Toledo, W. A. Chi-Chim, O. M. Torres-Chable, G. A. Cruz-Escalona, C. Machain-Williams, J. Mendez-Galvan, J. C. Tzuc-Dzul, and C. M. Baak-Baak. 2018. Laboratory evaluation of the Ile1, 016 mutation-effect on several life-history parameters of *Aedes aegypti*. Int. J. Mosq. Res. 5: 112–120.

Gorrochotegui-Escalante, N., C. Gomez-Machorro, S. Lozano-Fuentes, L. Fernandez-Salas, M. De Lourdes Munoz, J. A. Farfan-Ale, J. Garcia-Rejon, B. J. Beaty, and W. C. Black, 4th. 2002. Breeding structure of *Aedes aegypti* populations in Mexico varies by region. Am. J. Trop. Med. Hyg. 66: 213–222.

Hart, C. E., C. M. Roundy, S. R. Azar, J. H. Huang, R. Yun, E. Reynolds, G. Leal, M. R. Nava, J. Vela, P. M. Stark, et al. 2017. Zika Virus vector competency of mosquitoes, Gulf Coast, United States. Emerg. Infect. Dis. 23: 559–560.

Julian, E. G. –R., J. A Chan-Orilla, N. Cigarroa-Toledo, W. A. C. Chim, O. M. Torres-Chable, G. A. CruzEscalona, C. Machain-Williams, J. Mendez-Galvan, J. C. TzucDzul, and C. M. Baak-Baak. 2018. Laboratory evaluation of the Ile1, 016 mutation-effect on several life-history parameters of *Aedes aegypti*. Int. J. Mosq. Res. 5: 112–120.

Loroño-Pino, M. A., Y. N. Chan-Dzul, R. Zapata-Gil, C. Carrillo-Solís, A. Uitz-Mena, J. E. García-Rejón, T. J. Keefe, B. J. Beaty, and L. Eisen. 2014. Household use of insecticide consumer products in a dengue-endemic area in México. Trop. Med. Int. Health. 19: 1267–1275.

Martinez-Torres, D., F. Chandre, M. S. Williamson, F. Darriet, J. B. Bergé, A. L. Devonshire, P. Guillet, N. Pasteur, and D. Pauron. 1998. Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae s.s*. Insect Mol. Biol. 7: 179–184.

Martinez-Torres, D., C. Chevillon, A. Brun-Barale, J. B. Berge, N. Pasteur, and D. Pauron. 1999. Voltage-dependent Na+ channels in pyrethroid-resistant *Culex pipiens* L mosquitoes. Pest. Science. 55: 1012–1020.

Molly, C. R., and F. E. McKenzie. 2016. The contribution of agricultural insecticide use to increasing insecticide resistance in African malaria vectors. Malar. J. 15: 107.

Ndiath, M. O., S. Sougoufara, A. Gaye, C. Mazenot, L. Konate, O. Faye, O. Faye, C. Sokhna, and J. F. Trape. 2012. Resistance to DDT and pyrethroids and increased kdr mutation frequency in *An. gambiae* after the implementation of permethrin-treated nets in Senegal. PLoS One. 7: e31943.

Niang, e. l. H. A., L. Konaté, M. Diallo, O. Faye, and I. Dia. 2016. Patterns of insecticide resistance and knock down resistance (*kdr*) in malaria vectors *An. arabiensis*, *An. coluzzii* and *An. gambiae* from sympatric areas in Senegal. Parasit. Vectors. 9: 71.

Nkya, T. E., R. Poupardin, F. Laporte, I. Akhouayri, F. Mosha, S. Magesa, W. Kisinza, and J. P. David. 2014. Impact of agriculture on the selection of insecticide resistance in the malaria vector *Anopheles gambiae*: a multigenerational study in controlled conditions. Parasit. Vectors. 7: 480.

O'Reilly, A. O., B. P. Khambay, M. S. Williamson, L. M. Field, B. A. Wallace, and T. G. Davies. 2006. Modelling insecticide-binding sites in the voltage-gated sodium channel. Biochem. J. 396: 255–263.

Osta, M. A., Z. J. Rizk, P. Labbé, M. Weill, and K. Knio. 2012. *Insecticide resistance to organophosphates in Culex pipiens complex from Lebanon*. Parasit. Vectors. 5: 132.

Ponce, G., I. P. Sanchez, S. M. García, J. M. Torrado, S. Lozano-Fuentes, B. Lopez-Monroy, and A. E. Flores. 2016. First report of L1014F kdr mutation in *Culex quinquefasciatus* in Mexico. Insect Sci. 23: 829–834.

Rodriguez, M. M., J. A. Bisset, Y. De Armas, F. Ramos. 2005. Pyrethroid insecticide-resistant strain of *Aedes aegypti* from Cuba induced by deltamethrin selection. J. Am. Mosq. Cont. Assoc. 4: 437–445.

Saavedra-Rodriguez, K., L. Urdaneta-Marquez, S. Rajatileka, M. Moulton, A. E. Flores, I. Fernandez-Salas, J. Bisset, M. Rodriguez, P. J. McCall, M. J. Donnelly, et al. 2007. A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*. Insect Mol. Biol. 16: 785–798.

Saavedra-Rodriguez, K., M. Beaty, S. Lozano-Fuentes, S. Denham, J. Garcia-Rejon, G. Reyes-Solis, C. Machain-Williams, M. A. Loroño-Pino, A. Flores-Suarez, G. Ponce-Garcia, et al. 2015. Local evolution of pyrethroid resistance offsets gene flow among *Aedes aegypti* collections in Yucatan State, Mexico. Am. J. Trop. Med. Hyg. 92: 201–209.

Sarkar, M., A. Borkotoki, I. Baruah, I. K. Bhattacharyya, and R. B. Srivastava. 2009. Molecular analysis of knock down resistance (*kdr*) mutation and distribution of *kdr* genotypes in a wild population of *Culex quinquefasciatus* from India. Trop. Med. Int. Health. 14: 1097–1104.

Scott, J. G., M. H. Yoshimizu, and S. Kasai. 2015. Pyrethroid resistance in *Culex pipiens* mosquitoes. Pestic. Biochem. Physiol. 120: 68–76.

Singh, O. P., C. L. Dykes, G. Sharma, and M. K. Das. 2015. L1014F-kdr mutation in Indian *Anopheles subpictus* (Diptera: Culicidae) arising from two alternative transversions in the voltage-Gated sodium channel and a single PIRA-PCR for their detection. J. Med. Entomol. 52: 24–27.

Turell, M. J. 2012. Members of the *Culex pipiens* complex as vectors of viruses. j. Am. Mosq. Control Assoc. 28: 123–126.

Vazquez-Prokopec, G. M., A. Medina-Barreiro, A. Che-Mendoza, F. Dzul-Manzanilla, F. Correa-Morales, G. Guillermo-May, W. Bibiano-Marín, V. Uc-Puc, E. Geded-Moreno, J. Vadillo-Sánchez, et al. 2017. Deltamethrin resistance in *Aedes aegypti* results in treatment failure in Merida, Mexico. Plos Negl. Trop. Dis. 11: e0005656.

White, G. B. 1989. Lymphatic filariasis, pp 23–25 *In* World Health Organization: Geographical distribution of arthropod-borne diseases and their principal vectors. World Health Organization, Geneva, Switzerland. http://www.who.int/iris/handle/10665/60575

Williamson, M. S., D. Martinez-Torres, C. A. Hick, and A. L. Devonshire. 1996. Identification of mutations in the housefly para-type sodium channel gene associated with knockdown resistance (*kdr*) to pyrethroid insecticides. Mol. Gen. Genet. 252: 51–60.

Wondji, C. S., W. A. Priyanka De Silva, J. Hemingway, H. Ranson, and S. H. Parakrama Karunaratne. 2008. Characterization of knockdown resistance in DDT- and pyrethroid-resistant *Culex quinquefasciatus* populations from Sri Lanka. Trop. Med. Int. Health. 13: 548–555.

World Health Organization. 1998. Techniques to detect insecticide resistance mechanism. World Health Organization, Geneva, Switzerland. http://whqlibdoc.who.int/hq/1998/WHO_CDS_CPC_MAL_98.6.pdf?ua=1

Xu, Q., L. Tian, L. Zhang, and N. Liu. 2011. Sodium channel genes and their differential genotypes at the L-to-F *kdr* locus in the mosquito *Culex quinquefasciatus*. Biochem. Biophys. Res. Commun. 4: 645–649.