

## **Effect of Photoperiod On Permethrin Resistance In *Aedes aegypti***

Authors: Villanueva, O. Karina, Ponce, Gustavo, Lopez, Beatriz, Gutierrez, Selene M., Rodriguez, Iram P., et al.

Source: Journal of the American Mosquito Control Association, 32(4) : 308-314

Published By: The American Mosquito Control Association

URL: <https://doi.org/10.2987/16-6577.1>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

## EFFECT OF PHOTOPERIOD ON PERMETHRIN RESISTANCE IN *Aedes aegypti*

O. KARINA VILLANUEVA,<sup>1</sup> GUSTAVO PONCE,<sup>1</sup> BEATRIZ LOPEZ,<sup>1</sup> SELENE M. GUTIERREZ,<sup>1</sup> IRAM P. RODRIGUEZ,<sup>1</sup> GUADALUPE REYES,<sup>2</sup> KARLA J. SAAVEDRA,<sup>3</sup> WILLIAM C. BLACK IV,<sup>3</sup> JULIAN GARCIA,<sup>2</sup> BARRY BEATY,<sup>3</sup> LARS EISEN<sup>3</sup> AND ADRIANA E. FLORES<sup>1,4</sup>

**ABSTRACT.** Living organisms have been exposed to light–dark cycles that allowed them to adapt to different ecological niches. Circadian cycles affect hormone release, metabolism, and response to xenobiotic compounds. Current studies have shown that insect susceptibility to toxic agents depends on circadian cycles, mainly because the biochemical processes involved in detoxification and responses to oxidative stress are modulated by this process. The goal of this study was to determine the effect of photoperiod on resistance to permethrin in *Aedes aegypti*. Collections of *Ae. aegypti* from 4 locations in Yucatan, southern Mexico, were subjected to 2 different photoperiod schemes: dark (0 h light:24 h dark) and natural photoperiod (12 h light:12 h dark). The comparison of both photoperiods was evaluated with respect to permethrin resistance using bottle bioassays and by monitoring the possible mechanism related such as enzymatic activity and by the frequency of 2 knockdown resistance mutations in the voltage-dependent sodium channel gene (V1016I and F1534C). The susceptible strain was used as a reference. The mosquitoes in dark photoperiod showed a reduction in resistance to the pyrethroid. The  $\alpha$ -esterases and glutathione S-transferase enzymatic activities showed lower levels in the dark photoperiod, and the frequencies of V1016I knockdown resistance mutation showed significant difference between photoperiod schemes.

**KEY WORDS** *Aedes aegypti*, detoxifying enzymes, *kdr*, I1016, C1534, permethrin, photoperiod

### INTRODUCTION

Most living organisms, from bacteria to animals, are under the influence of the circadian rhythms. These are responsible for several mechanisms such as control of blood pressure, body temperature, hormone levels, and number of immune cells in the blood in animals and other systems in the organisms (Ishida et al. 1999). The rhythm is entrained by the environment, for example, through light stimuli (Schibler 2007). In several organisms, circadian rhythms are maintained through autoregulatory feedback loops in a central oscillator that are well conserved between insects and mammals (Giebultowicz 2001, Stanewsky 2003, Tobback et al. 2011).

The molecular machinery of the circadian clock has been extensively studied in the fruit fly, *Drosophila melanogaster* Meigen. There are several clock genes involved in the generation of the rhythmicity (Hardin 2006, Sandrelli et al. 2008). One major loop is that formed by the genes period (*per*), timeless (*tim*), Clock (*Clk*), and cycle (*cyc*) (Stanewsky 2002, Hardin 2006). Circadian genes have been cloned in species closely related to *D.*

*melanogaster*, such as *Anopheles gambiae* Giles (Holt et al. 2002) and *Aedes aegypti* (L.) (Nene et al. 2007).

*Aedes aegypti* is considered an urban mosquito and shows endophilic and anthropophilic behavior (Meireles-Filho and Kyriacou 2013), and it is the principal urban vector of dengue, chikungunya, Zika, and yellow fever viruses. Circadian rhythms in mosquitoes have been extensively studied, both in the wild and under laboratory conditions. Mosquitoes show a remarkable variation in locomotor activity behavior that can be affected by several factors such as nutrition and mating status, temperature, and light intensity (Clements 1999). The study of the circadian rhythms of arthropod vectors is of epidemiological relevance and important for disease control (Meireles-Filho and Kyriacou 2013).

Several studies have reported that some insects and mites display a circadian rhythm for susceptibility to toxic agents (Beck 1963, Cole and Adkisson 1964, Polcik et al. 1964). There is evidence that the effects of organophosphate, organochlorine, and pyrethroid pesticides on various pest insect species vary with the time of day during which they are applied (Sullivan et al. 1970, Eesa et al. 1995, Pszczolkowski and Dobrowolski 1999).

Although expression levels of detoxifying enzymes seem to remain constant until induced, microarray studies in several model species suggest that several xenobiotic metabolizing genes are expressed in daily rhythms such as glutathione S-transferase (GST) genes and cytochrome P450 superfamily of genes (Ptitsyn et al. 2011). Extensive studies of circadian gene expression reveal rhythms in the expression of multiple genes involved in the

<sup>1</sup> Universidad Autonoma de Nuevo Leon, Facultad de Ciencias Biologicas, Avenida Universidad s/n Cd. Universitaria, San Nicolas de los Garza, Nuevo Leon, 66455 Mexico.

<sup>2</sup> Laboratorio de Arbovirologia, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Calle 96 s/n, Paseo de Las Fuentes, Merida, Yucatan, CP 97225, Mexico.

<sup>3</sup> Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523.

<sup>4</sup> To whom correspondence should be addressed.

toxicological response of flies (Wijnen and Young 2006) and mammals (Yan et al. 2008).

Yang et al. (2010) showed that the knockdown time (KT<sub>50</sub>) in a selected strain population of *Ae. aegypti* was significantly longer in the light than in the dark phase, and that the mRNA level of *CYP9M9* (detoxification gene) was maximal in early scotophase, dropped to a minimum at midnight, and then slowly increased during the photophase. The existence of a clock control over mosquito sensitivity to permethrin was further indicated by reduced expression of *CYP9M9* and reduced mosquito resistance to permethrin after temporal silencing of the *per* gene.

Pyrethroids are the most common insecticides used for vector control. Permethrin was used in Mexico for >10 consecutive years (1999–2010) (Flores et al. 2013). Evidence of resistance to permethrin in *Ae. aegypti* populations in Mexico due to enzymatic mechanisms and knockdown-resistant (*kdr*) mutations in the voltage-gated sodium channel *para* gene have been reported (Flores et al. 2005, 2006, 2009, 2013; Saavedra-Rodríguez et al. 2007; Ponce et al. 2009; Siller et al. 2011; Aponte et al. 2013). Considering that this insecticide can induce oxidative stress (Vontas et al. 2001) and may be subject to circadian regulation (Krishnan et al. 2008), the objective of this study was to determine the effect of photoperiod on permethrin resistance and the mechanisms involved in *Ae. aegypti*.

## MATERIALS AND METHODS

### Mosquitoes

*Aedes aegypti* field populations were collected in 2012 from Yucatan State in southern Mexico; the localities sampled were: Vergel, Motul, Vergel III (20°57'08"N, 89°34'93"W), and Uman (20°53'77"N, 89°44'07"W). The New Orleans (NO) strain was used as a susceptible reference strain.

Laboratory colonies were established from larvae collected from natural breeding sites and maintained at 25 ± 4°C and 12:12 h light–dark (L:D) photoperiod. Pupae were placed in 250-ml flasks in cages (30 × 30 cm) until the adults emerged (parental generation). Sexing was performed by observing antennae having long hairs abundant in males unlike females. The male mosquitoes were fed with 10% sugar solution and the females on rats (*Rattus norvegicus* (Berkenhout)) as a source of blood. The females and males were placed in the same cage for mating and the production of eggs. These eggs corresponded to the F<sub>1</sub> generation, which were used in the light treatments and all bioassays. F<sub>1</sub> eggs were placed in plastic containers with dechlorinated water along with powdered liver protein as a food source provided daily for the subsequent larval stage. One group of adults was maintained in 12:12 h L:D photoperiod and another group to a complete dark period (0:24 h L:D), where cages were covered with a black voile fabric for the scotophase. All strains

were established and maintained at 25 ± 2°C and 70 ± 2% RH.

### Bioassays

Nonbloodfed F<sub>1</sub> females from field populations and susceptible NO strain (1–3 days old) were used in the bottle bioassays (Brogdon and McAllister 1998), in which a 250-ml Wheaton® bottle contained 1 ml of an acetone solution of technical-grade insecticide (ChemService, West Chester, PA). The bottle was capped and shaken to ensure uniform coverage and dried for an hour at room temperature. The insecticide tested was permethrin (40.1% *cis*–58.7% *trans*). Doses of AI (µg/bottle) were predetermined to obtain a range of knockdown and 24-h mortality rates from 0% to 99%. The number of different doses tested varied from 5 to 8 µg/bottle, with 3 repetitions per dose and 20 females per repetition.

The numbers of knocked-down mosquitoes were recorded every 10 min up to 1 h. After 1 h of exposure, all mosquitoes were gently transferred to recovery containers without insecticide and were offered a cotton ball soaked with a sugar solution. Mortality was recorded at 24 h. A dim red light (Omegon 33137; Nimax, Landsberg, Germany) was used to handle mosquitoes during the dark phase. Both bottles and recovery containers were kept at 24 ± 2°C and 70% RH.

We determined the concentration causing 50% knockdown (KC<sub>50</sub>) following 1 h of exposure. The concentration causing 50% mortality (LC<sub>50</sub>) was estimated 24 h after exposure to permethrin. The knockdown and lethal times (KT<sub>50</sub> and LT<sub>50</sub>) were also determined with the data obtained at KC<sub>50</sub> and LC<sub>50</sub> for each population/condition. The values of all parameters (KC<sub>50</sub>, LC<sub>50</sub>, KT<sub>50</sub>, and LT<sub>50</sub>) with 95% confidence limits were calculated using a quick calculator software program (<http://sourceforge.net/projects/irmaproj/files/>) with logistic regression.

### Enzyme assays

Sixty females from each population in each photoperiod treatment and NO reference strain were individually homogenized in 100 µl of 0.01 M potassium phosphate buffer, pH 7.2, and the homogenate resuspended in 2 ml of the same buffer. Next, 100 µl were transferred to microtiter plates; each sample was analyzed in triplicate on each plate. We then quantified the activities of α- and β-esterases, mixed-function oxidases (MFOs), and glutathione S-transferases (GSTs) according to the methods proposed by Brogdon (1989), Brogdon and Barber (1990), and Brogdon et al. (1997). Absorbance was measured using a UVM-340 microplate reader (ASYS Hitech GmbH, Eugendorf, Austria), and triplicate values were averaged. Protein concentration was determined using the method proposed by Brogdon (1984), and in some cases, it was necessary

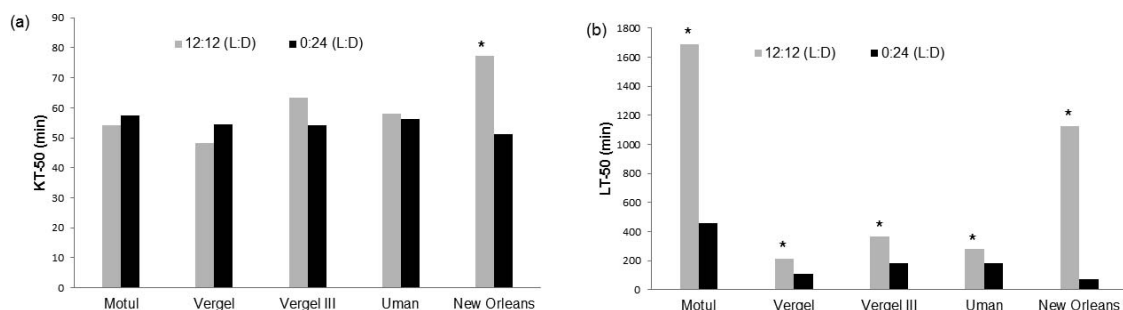


Fig. 1. Photoperiod change and permethrin resistance in *Aedes aegypti*. (a) The median knockdown time was significantly longer in the 12:12 h photoperiod only in the susceptible New Orleans strain. (b) Differences in the median lethal time in all mosquito strains showed that the susceptibility of *Ae. aegypti* was subject to change of photoperiod.

to dilute the homogenates because of variation in the size of the mosquitoes.

The data from each enzyme assay were analyzed by the nonparametric Kruskal–Wallis test ( $P < 0.05$ ), comparing the mean activity between the populations in each treatment.

### DNA isolation and genotype determination

DNA was isolated from each mosquito by the salting-out technique (Coen et al. 1982) and suspended in 50  $\mu$ l of buffer (10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid [EDTA, pH 8.0]) and stored at  $-70^{\circ}\text{C}$ . Genotypes at the I1016 locus were determined in a single-tube polymerase chain reaction (PCR) using the 2 different “allele-specific” primers and the reverse primer (Saavedra-Rodríguez et al. 2007) (Invitrogen, Carlsbad, CA). Products were subjected to agarose gel electrophoresis in a 1.5% (w/v) UltraPure™ agarose (Invitrogen) gel poured with Tris borate–EDTA (89 mM Tris-borate and 2 mM EDTA, pH 8.3). The DNA fragments were fractionated by electrophoresis for 30 min at 90 V alongside a 25-bp DNA ladder (Invitrogen).

For the F1534C mutation, allele-specific PCR was carried out in an Eco™ real-time thermal cycler (Illumina, San Diego, CA), to determine the genotypes by analysis of the denaturation curves following the protocols described by Yanola et al. (2011).

We included 3 controls for each PCR plate: a homozygous susceptible control (V1016/V1016 susceptible NO strain) and a homozygous resistant strain (I1016/I1016 resistant strain from Isla Mujeres, Mexico). For the F1534C mutation, we used a homozygous susceptible control (F1534/F1534 susceptible NO strain) and homozygous resistant strain (C1534/C1534 resistant strain).

The genotypic frequencies for the 1016 and 1534 loci were calculated by dividing the number of individuals with a given genotype by the total number of mosquitoes analyzed. Frequencies of field populations, as well as those subjected to 12:12 h or 0:24 h photoperiod, were compared.

## RESULTS

### KC<sub>50</sub>, LC<sub>50</sub>

Overall, slightly higher values of KC<sub>50</sub> were obtained with the 12:12 h photoperiod compared with 0:24 h for the populations tested including the NO strain. The KC<sub>50</sub> values for the populations of Motul and Vergel, under a 12:12 h photoperiod, were 0.44 and 0.53  $\mu\text{g}/\text{bottle}$ , respectively. The values obtained when females were exposed to a 0:24 h photoperiod were 0.51 and 0.67  $\mu\text{g}/\text{bottle}$ , respectively, but there was an overlap in the confidence intervals, indicating no significant difference between the KC<sub>50</sub> values between the 2 photoperiods. For Vergel III and Uman, KC<sub>50</sub> values for the individuals with a 12:12 h photoperiod were 11.99 and 13.75  $\mu\text{g}/\text{bottle}$ , respectively, which were significantly higher than the KC<sub>50</sub> values obtained with a 0:24 h photoperiod (9.35 and 10.50  $\mu\text{g}/\text{bottle}$ , respectively). For the susceptible NO strain, the KC<sub>50</sub> under a 12:12 h photoperiod was significantly higher (0.47  $\mu\text{g}/\text{bottle}$ ), compared with the KC<sub>50</sub> of 0.22  $\mu\text{g}/\text{bottle}$  obtained with 0:24 h (Table 1).

The LC<sub>50</sub> values under a 12:12 h photoperiod for all populations tended to be higher than those obtained with 0:24 h. However, the differences were significant for Vergel, Uman, and the susceptible NO strain. The LC<sub>50</sub> values in a 12:12 h photoperiod (0.59, 12.77, and 0.29  $\mu\text{g}/\text{bottle}$ , respectively) were significantly higher than the values obtained with a 0:24 h photoperiod (0.16, 7.9, and 0.18  $\mu\text{g}/\text{bottle}$ , respectively) (Table 1).

The median knockdown time was significantly longer in the 12:12 h photoperiod only in the susceptible NO strain (Fig. 1a). Differences in median lethal time in all mosquito strains showed that the susceptibility of *Ae. aegypti* was subject to circadian rhythm (Fig. 1b). This finding implied that *Ae. aegypti* was more resistant (based on LT<sub>50</sub>) during their active state in the 12:12 h photoperiod.

Mean values for  $\alpha$ - and  $\beta$ -esterases, MFOs, and GST of Motul, Vergel, Vergel III, Uman, and NO are shown in Table 2. The results indicated that  $\alpha$ -esterase and GST activities were significantly higher

Table 1. Knockdown concentrations (KC<sub>50</sub>) and lethal concentrations (LC<sub>50</sub>) of permethrin against *Aedes aegypti* strains at different photoperiods.<sup>1</sup>

Strain	12:12 h					0:24 h				
	N	KC <sub>50</sub> (95% CI)	b ± EE	LC <sub>50</sub> (95% CI)	b ± EE	N	KC <sub>50</sub> (95% CI)	b ± EE	LC <sub>50</sub> (95% CI)	b ± EE
Motul	475	0.4413 (0.4086–0.4765)	2.9234 (0.2716)	0.4868 (0.3952–0.5995)	2.0928 (0.2195)	352	0.5056 (0.4673–0.5471)	3.2046 (0.3904)	0.4352 (0.3943–0.4802)	1.092 (0.2670)
Vergel	539	0.5315 (0.4492–0.6287)	1.2551 (0.1176)	0.5907 (0.4969–0.7022)	0.7999 (0.1027)	538	0.6730 (0.5798–0.7810)	1.4096 (0.1276)	0.1627 (0.1097–0.2445)	1.186 (0.1134)
Vergel III	597	11.9950 (10.5729–13.6086)	1.7052 (0.1595)	7.3497 (6.4790–8.3370)	1.2601 (0.1142)	584	9.3523 (8.3788–10.4385)	2.0385 (0.1679)	6.6366 (5.5010–8.0080)	1.689 (0.1446)
Uman	471	13.7500 (13.3778–14.1328)	7.9282 (0.8034)	12.769 (12.3914–13.158)	7.4571 (0.8834)	657	10.496 (9.824–11.213)	2.7577 (0.2621)	7.8671 (7.1320–8.6780)	2.220 (0.2212)
New Orleans	477	0.4697 (0.4278–0.5159)	2.4543 (0.2792)	0.2875 (0.2475–0.3340)	1.5442 (0.1504)	477	0.2236 (0.2079–0.240)	3.099 (0.2912)	0.1767 (0.1679–0.1859)	5.409 (0.5536)

<sup>1</sup> CI, confidence interval; EE, 2 slope ± SE.

Table 2. Mean absorbance corrected by protein (± SD) values from the biochemical assays carried out on the 4 strains of *Aedes aegypti* and New Orleans strain with modified photoperiod.<sup>1</sup>

Strain	α-Esterases		β-Esterases		MFO		GST	
	12:12 h	0:24 h	12:12 h	0:24 h	12:12 h	0:24 h	12:12 h	0:24 h
Motul	0.731* (0.095)	0.579 (0.078)	0.998* (0.140)	0.993 (0.141)	0.212 (0.035)	0.228 (0.042)	0.083* (0.038)	0.056 (0.032)
Vergel	0.647* (0.033)	0.626 (0.044)	0.915 (0.057)	0.945 (0.090)	0.188 (0.022)	0.277 (0.038)	0.058* (0.014)	0.039 (0.011)
Vergel III	0.891* (0.078)	0.834 (0.088)	1.145 (0.131)	1.253 (0.107)	0.197 (0.026)	0.206 (0.018)	0.092* (0.022)	0.075 (0.029)
Uman	0.925* (0.099)	0.913 (0.060)	1.218* (0.129)	1.169 (0.126)	0.284* (0.045)	0.248 (0.033)	0.113* (0.039)	0.074 (0.025)
New Orleans	0.725* (0.056)	0.624 (0.042)	0.973 (0.098)	1.078 (0.157)	0.198* (0.032)	0.192 (0.036)	0.042* (0.011)	0.035 (0.007)

<sup>1</sup> GST, glutathione S-transferase; MFO, mixed-function oxidase.  
\* Mean values are significantly higher ( $P < 0.05$ ) compared with 0:24 h photoperiod.



Table 3. Genotype and allelic frequency of V1016I and F1534C of *Aedes aegypti* subjected to change in photoperiod.<sup>1</sup>

Strain–photoperiod	N	RR (I/I)	RS (I/V)	SS (V/V)	% RR alleles	% alleles I1016 (field population)	N	RR (C/C)	RS (C/F)	SS (F/F)	% RR	% alleles C1534 (field population)	N
Motul 12:12	15	7	0	8	46.7	48	25	3	5	7	36.7	60	14
Motul 0:24	20	6	5	9	42.5			6	6	6	50		
Vergel 12:12	15	1	0	14	6.7	79	50	3	6	6	40	100	30
Vergel 0:24	15	1	2	12	13.3			3	9	3	50		
Vergel III 12:12	20	20	0	0	100	75	14	20	0	0	100	54	25
Vergel III 0:24	18	7	0	11	38.9			18	0	0	100		
Uman 12:12	32	9	1	22	29.7	80	25	32	0	0	100	97	18
Uman 0:24	54	45	8	1	90.7			47	1	0	99.0		

<sup>1</sup> N, number of mosquitoes analyzed; SS, susceptible mosquitoes; RS, heterozygous; RR, homozygous mutant.

in the 12:12 h photoperiod compared with 0:24 h photoperiod in all populations tested. Table 3 shows the number of mosquitoes with each genotype and the I1016 and C1534 allele frequencies, along with 95% confidence interval for each photoperiod scheme. Overall, the proportion of RR (resistant homozygous) alleles for C1534 were higher in all populations analyzed, regardless of the photoperiod treatment (0:24 h and 12:12 h) compared with I1016. A Pearson  $\chi^2$  test to compare the frequencies of genotypes for I1016 and C1534 indicated significant differences between photoperiods in genotypes for I1016 ( $P < 0.001$ ) but not for C1534. The proportion of the C1534 genotype found in the strains in the 2 photoperiods was similar, suggesting that this particular *kdr* mutation does not appear to be mediated by the circadian cycle. These results support our hypothesis that susceptibility is linked to the circadian clock in *Ae. aegypti*. This study shows that esterase and the mutation V1016I have changes in their expression and frequency, respectively, but these mechanisms require further studies.

DISCUSSION

A number of factors can influence the susceptibility of insects to poisonous chemicals, for example, temperature, humidity, age, sex, and weight. Superimposed upon the effects of these factors are daily changes in physiology and behavior which may also influence insecticide susceptibility levels (Bainbridge et al. 1982). Circadian rhythms of mammalian sensitivity to drugs were first recorded by Halberg in 1960 and 1970 (Halberg 1960, 1970). At different times of the 24-h cycle, mice were found to react differently to equivalent doses of ethanol. Highest susceptibility was recorded (in 12:12 h L:D) toward the end of the light period and lowest susceptibility close to midnight. A circadian susceptibility–resistance cycle to ethanol persisted in mice kept in continuous darkness for several days. The data reported in this paper in *Ae. aegypti* support our objective of a link between the photoperiod and insecticide resistance. The following points support this hypothesis: first, the results of knockdown

resistance (RRKC<sub>50</sub>) and postrecovery at 24 h (RRLC<sub>50</sub>) obtained in our study are consistent with those reported by Yang et al. (2010), who proposed that mosquitoes are more susceptible during the dark phase of the photoperiod and show less sensitivity during the light phase, because this is considered the time of their active state. Second, enzyme data support previous studies in which the expression was found to be regulated by circadian clocks in *Ae. aegypti* (Ptitsyn et al. 2011) and for GST in *An. gambiae* (Rund et al. 2013).

The results support our hypothesis that susceptibility is linked to photoperiod effect in *Ae. aegypti*. This study shows that esterase and the mutation V1016I have changes in their expression and frequency, respectively. This study highlights the potential of chronotoxicity (Pszczolkowski and Dobrowolski 1999) to improve the effectiveness of chemical pest management programs.

ACKNOWLEDGMENTS

This work was supported by Fogarty Training Grant 2D43TW001130-08 “Training in Dengue Prevention and Control.” The study was also supported by the National Institutes of Health/ National Institute of Allergy and Infectious Diseases (NIH/NIAID; International Collaborations in Infectious Disease Research Program U01-AI-088647).

REFERENCES CITED

Aponte HA, Penilla RP, Dzul-Manzanilla F, Che-Mendoza A, Lopez AD, Solis F, Manrique-Saide P, Ranson H, Lenhart A, McCall PJ, Rodriguez AD. 2013. The pyrethroid resistance status and mechanisms in *Aedes aegypti* from the Guerrero state, Mexico. *Pestic Biochem Physiol* 107:226–234.

Bainbridge CA, Margham P, Thomas M. 1982. Diurnal fluctuations in susceptibility to insecticides in several strains of the yellow fever mosquito (*Aedes aegypti* L). *Pestic Sci* 13:92–96.

Beck SD. 1963. Physiology and ecology of photoperiodism. *Bull Entomol Soc Am* 9:8–16.

- Brogdon WG. 1984. Mosquito protein microassay. I. Protein determinations from small portions of single-mosquito homogenates. *Comp Biochem Physiol B* 79:457–459.
- Brogdon WG. 1989. Biochemical resistance detection: an alternative to bioassay. *Parasitol Today* 5:56–60.
- Brogdon WG, Barber AM. 1990. Microplate assay of glutathione S-transferase activity for resistance detection in single-mosquito triturates. *Comp Biochem Physiol* 96:339–342.
- Brogdon WG, McAllister JC. 1998. Simplification of adult mosquito bioassays through use of time mortality determinations in glass bottles. *J Am Mosq Control Assoc* 14:59–164.
- Brogdon WG, McAllister JC, Vulule J. 1997. Heme peroxidase activity measured in single mosquitoes identifies individuals expressing an elevated oxidase for insecticide resistance. *J Am Mosq Control Assoc* 13:233–237.
- Clements AN. 1999. *The biology of mosquitoes*. Volume 2. Wallingford, United Kingdom: CABI.
- Coen E, Strachan T, Dover G. 1982. Dynamics of concerted evolution of ribosomal DNA and histone gene families in the melanogaster species subgroup of *Drosophila*. *J Mol Biol* 158:17–35.
- Cole CL, Adkisson PL. 1964. Daily rhythm in the susceptibility of an insect to a toxic agent. *Science* 144:1148–1149.
- Eesa NM, Cutkomp KL. 1995. Pesticide chronotoxicity to insects and mites: an overview. *J Islamic Acad Sci* 8:21–28.
- Flores AE, Albeldaño WV, Fernandez IS, Badii MH, Loaiza H, Ponce GG, Lozano SF, Brogdon WG, Black WC, Beaty BJ. 2005. Elevated  $\alpha$ -esterase levels associated with permethrin tolerance in *Aedes aegypti* (L.) from Baja California, Mexico. *Pest Biochem Physiol* 82:66–78.
- Flores AE, Grajales JS, Fernandez IS, Ponce GG, Loaiza H, Badii MH, Lozano SF, Brogdon WG, Black WC, Beaty BJ. 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 AE, Ponce G, Silva BG, Gutierrez SM, Bobadilla C, Lopez B, Mercado R, Black WC 4th. 2013. Wide spread cross resistance to pyrethroids in *Aedes aegypti* (Diptera: Culicidae) from Veracruz state Mexico. *J Econ Entomol* 106:959–969.
- Flores AE, Reyes G, Fernandez IS, Sanchez RFJ, Ponce GG. 2009. Resistance to permethrin in *Aedes aegypti* (L.) in northern Mexico. *Southwest Entomol* 34:167–177.
- Giebultowicz JM. 2001. Peripheral clocks and their role in circadian timing: insights from insects. *Philos Trans R Soc Lond B Biol Sci* 356:1791–1799.
- Halberg F. 1960. Symposium on some current research methods and results with special reference to the central nervous system—physiopathologic approach. *Am J Ment Defic* 65:156–171.
- Halberg F. 1970. Temporal coordination of physiological function. *Cold Spring Harbor Symp Quant Biol* 25:289–310.
- Hardin PE. 2006. Essential and expendable features of the circadian timekeeping mechanism. *Curr Opin Neurobiol* 16:686–692.
- Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, Nusskern DR, Wincker P, Clark AG, Ribeiro JM, Wides R, Salzberg SL, Loftus B, Yandell M, Majoros WH, Rusch DB, Lai Z, Kraft CL, Abril JF, Anthouard V, Arensburger P, Atkinson PW, Baden H, de Berardinis V, Baldwin D, Benes V, Biedler J, Blass C, Bolanos R, Boscus D, Barnstead M, Cai S, Center A, Chaturverdi K, Christophides GK, Chrystal MA, Clamp M, Cravchik A, Curwen V, Dana A, Delcher A, Dew L, Evans CA, Flanigan M, Grundschober-Freimoser A, Friedli L, Gu Z, Guan P, Guigo R, Hillenmeyer ME, Hladun SL, Hogan JR, Hong YS, Hoover J, Jaillon O, Ke Z, Kodira C, Kokoza E, Koutsos A, Letunic I, Levitsky A, Liang Y, Lin JJ, Lobo NF, Lopez JR, Malek JA, McIntosh TC, Meister M, Miller J, Mobarry C, Mongin E, Murphy SD, O'Brochta DA, Pfannkoch C, Qi R, Regier MA, Remington K, Shao H, Sharakhova MV, Sitter CD, Shetty J, Smith TJ, Strong R, Sun J, Thomasova D, Ton LQ, Topalis P, Tu Z, Unger MF, Walenz B, Wang A, Wang J, Wang X, Woodford KJ, Wortman JR, Wu M, Yao A, Zdobnov EM, Zhang H, Zhao Q, Zhao S, Zhu SC, Zhimulev I, Colluzi M, della Torre A, Roth CW, Louis C, Kalush F, Mural RJ, Myers EW, Adams MD, Smith HO, Broder S, Gardner MJ, Fraser CM, Birney E, Bork P, Brey PT, Venter JC, Weissenbach J, Kafatos FC, Collins FH, Hoffman SL. 2002. The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science* 298:129–149.
- Ishida N, Kaneko M, Allada R. 1999. Biological clocks. *Proc Natl Acad Sci USA* 96:8819–8820.
- Krishnan N, Davis AJ, Giebultowicz JM. 2008. Circadian regulation of response to oxidative stress in *Drosophila melanogaster*. *Biochem Biophys Res Commun* 374:299–303.
- Meireles-Filho AC, Kyriacou CP. 2013. Circadian rhythms in insect disease vectors. *Mem Inst Oswaldo Cruz* 108:48–58.
- Nene V, Wortman JR, Lawson D, Haas B, Kodira C, Tu ZJ, Loftus B, Xi Z, Megy K, Grabherr M, Ren Q, Zdobnov EM, Lobo NF, Campbell KS, Brown SE, Bonaldo MF, Zhu J, Sinkins SP, Hogenkamp DG, Amedeo P, Arensburger P, Atkinson PW, Bidwell S, Biedler J, Birney E, Bruggner RV, Costas J, Coy MR, Crabtree J, Crawford M, Debruyne B, Decaprio D, Eiglmeier K, Eisenstadt E, El-Dorri H, Gelbart WM, Gomes SL, Hammond M, Hannick LI, Hogan JR, Holmes MH, Jaffe D, Johnston JS, Kennedy RC, Koo H, Kravitz S, Kriventseva EV, Kulp D, Labutti K, Lee E, Li S, Lovin DD, Mao C, Mauceli E, Menck CF, Miller JR, Montgomery P, Mori A, Nascimento AL, Naveira HF, Nusbaum C, O'leary S, Orvis J, Perteira M, Quesneville H, Reidenbach KR, Rogers YH, Roth CW, Schneider JR, Schatz M, Shumway M, Stanke M, Stinson EO, Tubio JM, Vanze JP, Verjovski-Almeida S, Werner D, White O, Wyder S, Zeng Q, Zhao Q, Zhao Y, Hill CA, Raikhel AS, Soares MB, Knudson DL, Lee NH, Galagan J, Salzberg SL, Paulsen IT, Dimopoulos G, Collins FH, Birren B, Fraser-Liggett CM, Severson DW. 2007. Genome sequence of *Aedes aegypti*, a major arbovirus vector. *Science* 316:1718–1723.
- Polcik B, Nowosielski JW, Naegele JA. 1964. Daily sensitivity rhythm of the two-spotted spider mite, *Tetranychus urticae*, to DDVP. *Science* 145:405–406.
- Ponce G, Flores A, Fernandez I, Saavedra K, Reyes G, Lozano S, Bond G, Casas M, Ramsey, Garcia J, Dominguez M, Ranson H, Hemingway J, Eisen L, Black WC 4th. 2009. Recent rapid rise of a permethrin knock down resistance allele in *Aedes aegypti* in México. *PLoS Negl Trop Dis* 3:531–560.

- Pszczolkowski MA, Dobrowolski M. 1999. Circadian dynamics of locomotor activity and deltamethrin susceptibility in the pine weevil, *Hylobius abietis*. *Phytoparasitica* 27:19–25.
- Ptitsyn AA, Reyes-Solis G, Saavedra-Rodríguez K, Betz J, Suchman EL, Carlson JO, Black WC 4th. 2011. Rhythms and synchronization patterns in gene expression in the *Aedes aegypti* mosquito. *BMC Genomics* 12:1–16.
- Rund SS, Gentile JE, Duffield GE. 2013. Extensive circadian and light regulation of the transcriptome in the malaria mosquito *Anopheles gambiae*. *BMC Genomics* 14:1–19.
- Saavedra-Rodríguez K, Urdaneta-Marquez L, Rajatileka S, Moulton M, Flores AE, Fernandez-Salas I, Bisset J, Rodriguez M, McCall PJ, Donnelly MJ, Ranson H, Hemingway J, Black WC 4th. 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.
- Sandrelli F, Costa R, Kyriacou CP, Rosato E. 2008. Comparative analysis of circadian clock genes in insects. *Insect Mol Biol* 17:447–463.
- Schibler U. 2007. The daily timing of gene expression and physiology in mammals. *Dialogues Clin Neurosci* 9:257–272.
- Siller Q, Ponce G, Lozano S, Flores AE. 2011. Update on the frequency of Ilde1016 mutation in voltage-gated channel gene of *Aedes aegypti* in Mexico. *J Am Mosq Control Assoc* 27:357–362.
- Stanewsky R. 2002. Clock mechanism in *Drosophila*. *Cell Tissue Res* 309:11–26.
- Stanewsky R. 2003. Genetic analysis of the circadian system in *Drosophila melanogaster* and mammals. *J Neurobiol* 54:111–147.
- Sullivan WN, Cawley B, Hayes DK, Rosenthal J, Halberg F. 1970. Circadian rhythm in susceptibility of house flies and Madeira cockroaches to pyrethrum. *J Econ Entomol* 63:159–163.
- Tobback J, Boerjan B, Vandermissen HP, Huybrechts R. 2011. The circadian clock genes affect reproductive capacity in the desert locust *Schistocerca gregaria*. *Insect Biochem Mol Biol* 41:313–321.
- Vontas JG, Small GJ, Hemingway J. 2001. Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens*. *Biochem J* 357:65–72.
- Wijnen H, Young MW. 2006. Interplay of circadian clocks and metabolic rhythms. *Annu Rev Genet* 40:409–448.
- Yan J, Wang H, Liu Y, Shao C. 2008. Analysis of gene regulatory networks in the mammalian circadian rhythm. *PLoS Comput Biol* 4:e1000193.
- Yang YY, Liu Y, Teng HJ, Sauman L, Sehnael F, Lee HJ. 2010. Circadian control of permethrin-resistance in the mosquito *Aedes aegypti*. *J Insect Physiol* 56:1219–1223.
- Yanola J, Somboon P, Walton C, Nachaiwieng W, Somwang P, Prapanthadara L. 2011. High-throughput assays for detection of the F1534C mutation in the voltage-gated sodium channel gene in permethrin-resistant *Aedes aegypti* and the distribution of this mutation throughout Thailand. *Trop Med Int Health* 16:501–509.