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LIFE HISTORIES AND OTHER BIOLOGICAL CHARACTERISTICS ENABLING THE ESTABLISHMENT OF *AEDES ALBOPICTUS* IN THE SAN GABRIEL VALLEY, CALIFORNIA

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ABSTRACT. Since *Aedes albopictus* was discovered in 2011 in the San Gabriel Valley it has become widespread despite the "harsh" environmental conditions and intense efforts to control or eliminate it. Species introduced into a new area may survive, thrive, or disappear depending on whether its new environment is suitable. The San Gabriel Valley Mosquito and Vector Control District expended considerable resources from 2011 to 2015 to eradicate this invasive species or, at a minimum, control and manage its spread. Despite the intense effort, the distribution of *Ae. albopictus* steadily expanded. Over those 5 years this increase shifted from a geometric to exponential pattern. What enabled *Ae. albopictus* to survive initially, become established, and then expand their distribution when ecological conditions in southern California were considered hostile for this invasive species? This study explores several biological characteristics including skip oviposition, installment egg hatching, and variable larval development that may have helped *Ae. albopictus* flourish in its new environment.

KEY WORDS *Aedes albopictus,* Asian tiger mosquito, exotic pests, invasion biology, invasive species, life histories, succession

INTRODUCTION

Aedes albopictus (Skuse) is a highly successful invasive mosquito species (Hawley 1988, Moore et al. 1988). Its success at interspecific competition has allowed it to displace other species of mosquitoes, especially *Ae. aegypti* (L.) in many localities in the United States (Juliano and Lounibos 2005). *Aedes albopictus* (Asian tiger mosquito) thrives in locations with high humidity and warm temperatures but can survive in imperfect locations if a variety of sugar sources are available (Hawley 1988). It is not obvious why this mosquito has been able to adapt to the Mediterranean climate of southern California and the San Gabriel Valley to the point where eradication has proven impossible (Fujioka et al. 2012, Wekesa et al. 2014). Barker and others (2015) modeled the process of successful colonization of suitable continuous yards and/or like properties by this mosquito in the area. Previous introductions of *Ae. albopictus* into California were not successful due to many factors, and great efforts were made to eradicate them when they were found (Linthicum et al. 2003, Madon et al. 2003). Predictions in Moore (1999) considered the establishment of this mosquito in California extremely unlikely to impossible due to mostly winter precipitation unsuitable for the biology and behavior of *Ae. albopictus* despite the role of human activities.

What biological abilities does this mosquito of temperate origin (Zhong et al. 2013) possess so it rapidly adapted to its new environment in southern California? *Aedes* spp. exhibit variable oviposition patterns, e.g., skip oviposition, where gravid females rarely oviposit their entire clutch of eggs at the same oviposition site (Mogi 1982, Colton et al. 2003). *Aedes albopictus* and *Ae. aegypti* have demonstrated this phenomenon both in the lab and in the field. In addition, *Ae. albopictus* from tropical and temperate environments has been shown to lay eggs with different levels of diapause with varying rates of this phenotype (Hawley 1988, Toma et al. 2003, Vitek and Livdahl 2006, Urbanski et al. 2010). *Aedes albopictus* lay eggs that hatch at different rates. Research has suggested that photoperiodic diapause may allow *Ae. albopictus* to colonize temperate environments (Hawley 1988, Toma et al. 2003), such as the San Gabriel Valley. *Aedes albopictus* is such a successful invasive species because of its behavior.

In other parts of the world *Ae. albopictus* lay their eggs in multiple microhabitats (Juliano and Lounibos 2005), a strategy that allows for greater survival. It acclimates to areas by changing behavior over generations to take advantage of the resources at hand (Mogi 1982). This article explores a biological basis on how *Ae. albopictus* has been able to invade, establish, and thrive in the San Gabriel Valley and southern California. We look at skip oviposition, installment egg hatching, and seasonal larval development time to explain why this mosquito has done so well.

MATERIAL AND METHODS

This study was conducted in 2014 in the city of El Monte and additional nearby cities where infestations

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of *Ae. albopictus* were confirmed and no other member of the invasive *Aedes*, *Ae. aegypti* and *Ae. notoscriptus* (Skuse), had been identified in the area. In addition, many eggs hatched and larvae raised to adults never showed mixed species throughout the study period. All life stages were collected for testing the biological phenomena of skip oviposition, installment egg hatching, and seasonal larval development.

Site description and sampling

All areas infested by *Ae. albopictus* during 2014 were potential sources of eggs used in this study. Locations confirmed as infested by *Ae. albopictus* in 2014 and prior years included the city of El Monte, where *Ae. albopictus* was discovered initially (Fujioka et al. 2012), Arcadia and Duarte (Wekesa et al. 2014), and Baldwin Park, Bradbury, Irwindale, La Puente, Monrovia, Monterey Park, Rosemead, and Temple City (Brisco et al. 2015). At the end of 2016, all 23 cities except Pomona in the San Gabriel Valley Mosquito and Vector Control District's jurisdiction were infested with *Ae. albopictus*. The city of Pomona was eventually confirmed infested in June 2017. Properties that were infested with *Ae. albopictus* were identified by either intensive door-to-door inspections, trapping, oviposition, or service requests or by a combination of these activities. Stages of *Ae. albopictus* (eggs, larvae, pupae, and adults) that were present were confirmed by visual observation or by using a variety of traps (Fujioka et al. 2012, Wekesa et al. 2014, Brisco et al. 2015). Oviposition cups (ovicups) containing water and strips of seed germination paper (oviposition strips) were placed during the study period throughout the cities positive for *Ae. albopictus*. Ovicups were placed in areas known to have *Ae. albopictus* that were easily accessible, were less likely to be vandalized or disturbed, had vegetation conducive for resting, and had shade, which was more likely to attract gravid female mosquitoes (Li et al. 2014). Special attention was given to ensure that oviposition strips were not destroyed by garden snails or ovicups not tipped over.

Ovicups were inspected once a week by staff. All oviposition strips that were positive for *Ae. albopictus* eggs were labeled and brought back to the laboratory. Ovicups that required maintenance were cleaned, fresh water was added, and the oviposition strip was replaced.

Egg hatching

The eggs of *Ae. albopictus* that were found on oviposition strips were counted and examined for conditions such as deflation (these were deemed nonviable) and whether they previously hatched. Previously hatched eggs were excluded from counts except when larvae were present. *Aedes albopictus* eggs deemed viable were initially held in resealable plastic bags for 24 h and before flooding. Oviposition strips that showed evidence of active hatching were immediately flooded. Mosquito eggs were flooded by submerging oviposition strips in 120 ml of hay infusion (brewer's yeast and ground alfalfa pellets, diluted by dechlorinated water at a ratio of 1:5) in 250 ml plastic cups. In the months of June through September the collected *Aedes* eggs on oviposition strips were so abundant that after bringing them into the laboratory they were placed in a box and 1 out of every 10 oviposition strips were randomly flooded to determine the percentage of egg hatching.

Duration of larval development

To determine how long eggs took to hatch into larvae once flooded and develop into pupa and adults, the eggs collected during door-to-door inspections in the field were divided into two sets. One set of eggs was held in the field, and the other set was brought into the laboratory. The eggs in both treatments were submerged in 100 ml of 1:5 hay infusion held in 266 ml clear plastic cups (Smart and Final, Commerce, CA). The date when eggs were flooded was considered day 0, and every 2 days thereafter the number of unhatched eggs, number of larvae in each instar, and number of pupae or adults were recorded. This monitoring and recording continued until all eggs hatched or the study ended. Since diapausing eggs routinely do not hatch until conditions are favorable, we used the procedure of Mogi (1982) and dried unhatched eggs for 2 days, then resubmerged them in the same solution for 1 wk and repeated this process until either no eggs hatched or no eggs were left to hatch on the oviposition strip.

Field-held eggs and larvae: The eggs and larvae collected and held in the field were collected between January 2014 and May 2014 from yard containers, not from oviposition cups. The set of eggs and early stage larvae held in the field were counted and flooded. They were transferred into 250 ml plastic cups with 120 ml solution of 1:5 hay infusion, placed inside mosquito cages, and held at the same property for the duration of the study. These samples of eggs and larvae were checked and if hatched the stages recorded every 2 days until all the larvae developed into pupae and adults.

Laboratory-held eggs: The eggs brought into the laboratory were collected from oviposition cups. These eggs were similarly treated as the eggs that were collected in the field, and the number of larvae in each instar was recorded every 2 days. The laboratory hatched eggs were observed through larval stages and were disposed after they reached 4th-stage larvae.

Data analysis

The date when eggs were flooded was considered day 0 and were successively (day 0, 2, 4, 6, etc.) counted until eggs hatched or the study ended. The development times from day 0 to when larvae reached 4th instar were counted and analyzed over winter, spring, summer, and fall of 2014. Throughout the year, the hatch rates for each collection were

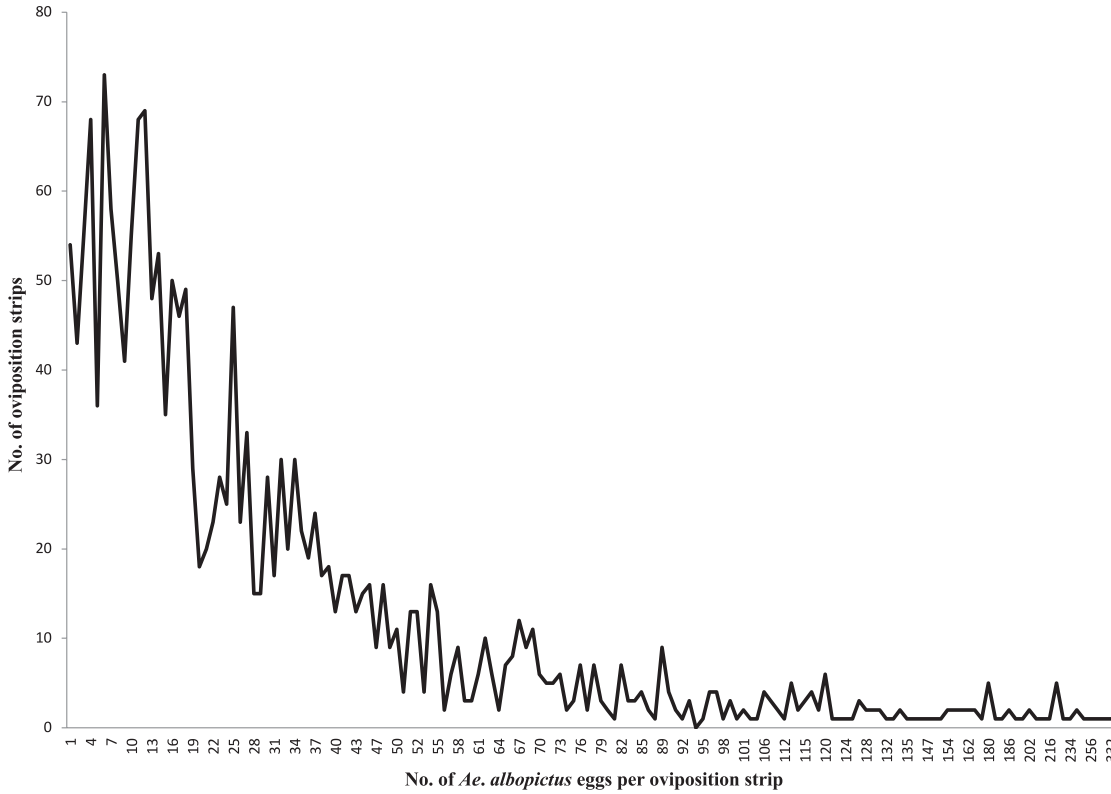

Fig. 1. The frequency of *Ae. albopictus* eggs collected on 176 positive-oviposition strips in 2014 in San Gabriel Valley with a mean of 34 eggs per week, range of 1 to 355 eggs, and a mode of ≤17 eggs per oviposition strip per wk.

calculated once each cohort had either successfully reached 100% hatch rate or completely stopped hatching out after repeated drying and flooding. Development times were analyzed once all larvae in each cohort had reached the 4th instar. The percentage of eggs hatching and the number of days for eggs to develop to stage 4 larvae were averaged by season and any life history patterns documented. To determine the average development times of this mosquito, only the first wave of hatched larvae were analyzed.

RESULTS

A total of 298 ovicups were placed in the field during the study period. One-hundred-seventy-six ovicups/traps collected 63,204 eggs.

Skip oviposition

Eggs from the oviposition cups were analyzed to determine whether *Ae. albopictus* deposited a full batch of eggs at each gonotrophic cycle. The number of eggs deposited on each oviposition strip per ovicup were plotted against the number of times a strip collected that amount of eggs (Fig. 1). The distribution of eggs per oviposition strip showed several peaks of egg-laying clusters. Of the 176 positive ovicups, the total number of eggs collected per ovicup ranged from 1 to 355 eggs per wk, the mode was less than 17 eggs per oviposition strip, and the mean was 34 *Ae. albopictus* eggs per wk.

Egg hatching and hatching rates

In this study, we used 3,362 eggs from 136 oviposition strips. The hatching of cohort of eggs varied widely throughout the year. Only 17% of eggs collected in late fall of 2013 and winter of 2014 hatched. In contrast 79% of spring 2014 eggs hatched, 68% of summer hatched, and 69% of fall 2014 eggs hatched (Fig. 2).

Eggs that did not hatch after the initial flooding hatched after repeated drying and reflooding. Cumulative hatch rates for cohorts of eggs that were repeatedly dried and reflooded reached 70% at 40 days of observation. Most eggs hatched in the first 2 wk of flooding with low-level hatching continued for up to 5 more wk. This observation was more pronounced in winter and spring than in summer and fall (Fig. 3).

Variable developmental times

Aedes albopictus eggs that were found in containers and transferred into mosquito cages in the field

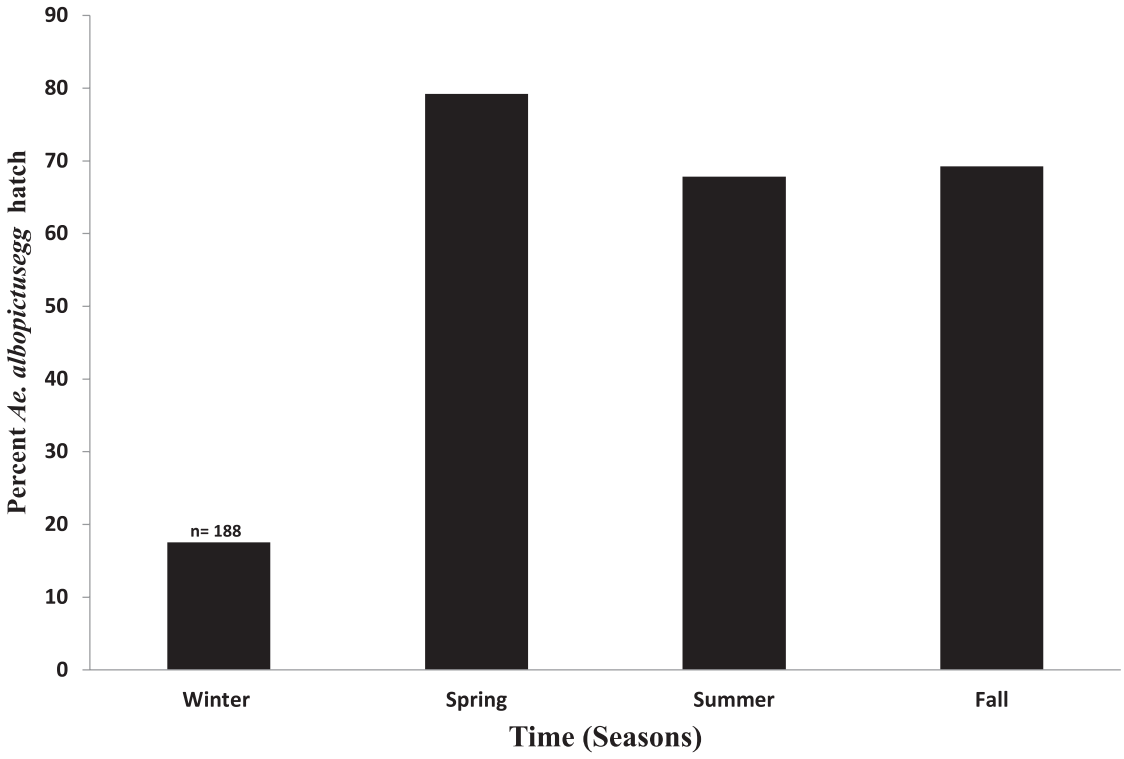

Fig. 2. The percentage of *Ae. albopictus* eggs hatching for each season of 2014 from a total of 3,362 eggs collected on 176 positive-oviposition strips.

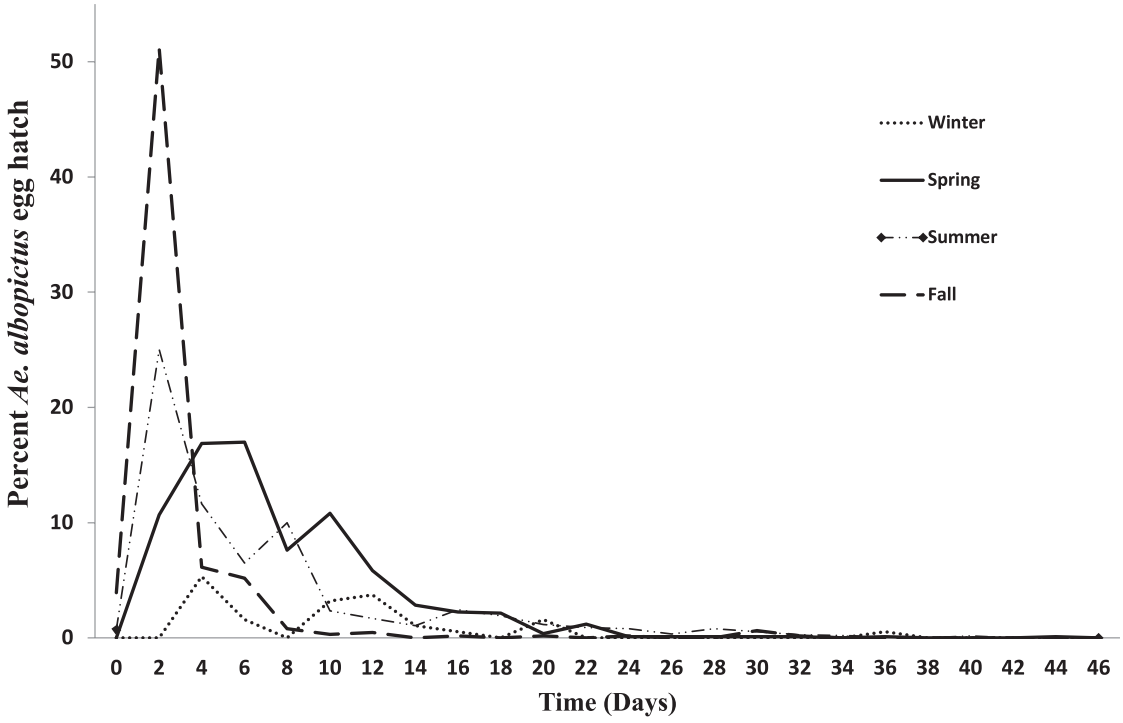

Fig. 3. The extended seasonal frequency of *Ae. albopictus* eggs hatching over 7 wk in 2014.

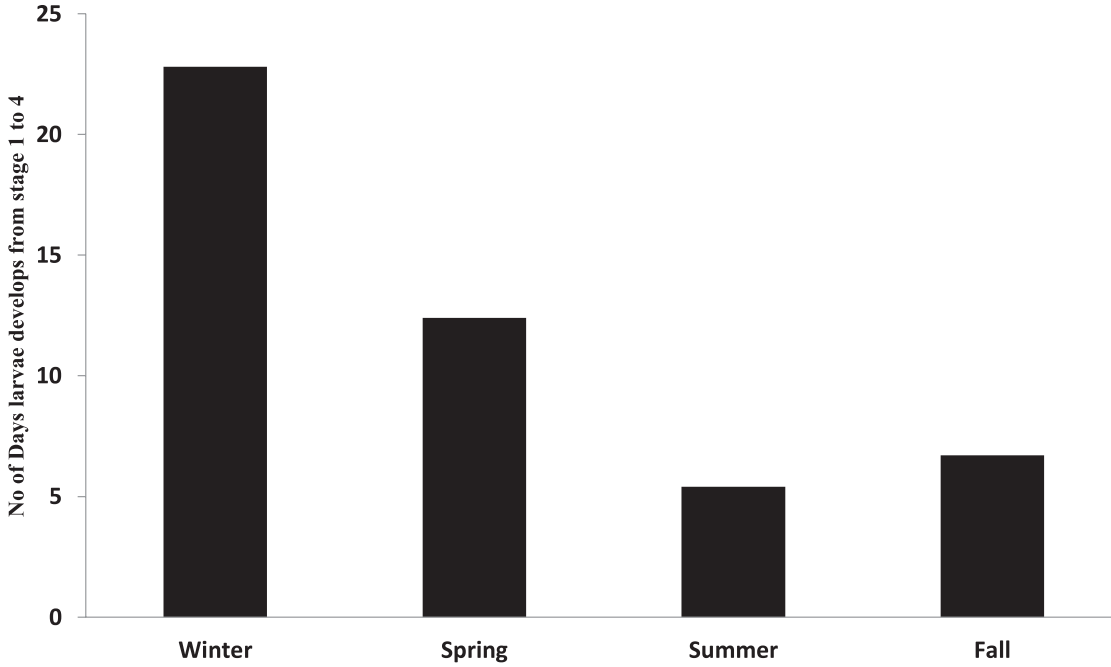

Fig. 4. The number of days for *Ae. albopictus* 1st-stage larvae to reach 4th stage during the 4 seasons of 2014.

were flooded and observed. These eggs collected in the field were observed only during the winter of 2014. A total of 183 *Ae. albopictus* eggs were collected, and only 6% of them hatched after 7 wk of observation; it took 48 days for 3% of the hatched eggs as larvae to grow from 1st to 4th instar. Overall analysis of the developmental times of *Ae. albopictus* once eggs hatched into 1st instar showed that it took about 23 days for such 1st instars to develop to 4th instars in the winter months as compared to 12 days in spring, 5 days in summer, and 7 days in fall (Fig. 4).

DISCUSSION

The mode of eggs laid per oviposition strip was less than 17 eggs (Fig. 1), implying that when most *Ae. albopictus* females attempted to oviposit, 1–17 eggs were laid. Previous reports indicated that female *Ae. albopictus* laid 42–88 eggs in their first gonotrophic cycle, and 40–80 eggs in subsequent cycles (Hawley 1988). In the present study a mean of 34 eggs were laid, but the majority of the oviposition strips had 1–17 eggs, implying that *Ae. albopictus* females did not deposit all of their eggs at 1 oviposition site. This is evidence of a skip oviposition behavior occurring in the local *Ae. albopictus* populations (Kuno 2012, Colton et al. 2013).

The staggered hatching observed within cohorts of *Ae. albopictus* gives the progeny in a container an advantage by reducing competition between conspecific larvae and with other mosquito species whose eggs all hatch within a few days. This ensures that the invading species can thrive from one season to the next (Juliano and Lounibos 2005). Furthermore, installment egg hatching undermines control efforts because eggs remain viable on dry containers, thus requiring multiple larvicidal applications to eliminate a single cohort of mosquitoes (Peacock et al. 1988). Finally, the longer developmental time for *Ae. albopictus* observed during the winter season (5 times longer than summer cohorts) allows *Ae. albopictus* to survive as larvae until favorable conditions for pupal and adult survival are present in the spring season.

Aedes albopictus in the San Gabriel Valley exhibit several biological characteristics that allow them to thrive despite their temperate origin (Zhong et al. 2013). Skip oviposition, variable larval development, and installment egg hatching or what could be discerned as egg diapause (Hanson and Craig 1994) all contribute to their ability to survive in a new ecosystem. These behaviors may be responsible for the establishment of *Ae. albopictus* in the San Gabriel Valley and the reason for the inability to have a prolonged impact in suppressing them.

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REFERENCES CITED

Barker CM, Montecito D, Kluh S, Fujioka KK, Morales H, Brisco A, Wekesa JW. 2015. Modeling control of the highly invasive mosquito, *Aedes albopictus*. *Proc Papers Mosq Vector Control Assoc Calif* 83:22–23.

Brisco A, "The Albo Crew," Fujioka KK, Wekesa JW. 2015. Eradication of *Aedes albopictus* in the San Gabriel Valley, California is doubtful using current methodologies. *Proc Papers Mosq Vector Control Assoc Calif* 83:22–23.

Colton YM, Chadee DD, Severson DW. 2003. Natural "skip oviposition" of the mosquito *Aedes aegypt*i as evidenced by codominant genetic markers. *Med Vet Entomol* 18:195–204.

Fujioka KK, Middleton KM, Sorvillo TE, Brisco MA, Tanaka ME, Mitchel ME. 2012. Discovery of *Aedes albopictus* (Skuse) in the City of El Monte and the initial response. *Proc Papers Mosq Vector Control Assoc Calif* 80:27–29.

Hanson SM, Craig GB Jr. 1994. Cold acclimation, diapause, and geographic origin affect cold hardiness in eggs of *Ae. albopictus* (Diptera: Culicidae). *J Med Entomol* 31:192–201

Hawley WA. 1988. The biology of *Aedes albopictus*. *J Am Mosq Control Assoc Suppl* 4:1–39.

Juliano SA, Lounibos PL. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecol Lett* 8:558–574

Kuno G. 2012. Revisiting Houston and Memphis: the background histories behind the discovery of the infestation by *Aedes albopictus* (Diptera: Culicidae) in the United States and their significance in the contemporary research. *J Med Entomol* 49:1163–1176.

Li Y, Kamara F, Zhou G, Puthiyakunnon S, Li C, Liu Y, Zhou Y, Yao L, Yan G, Chen X. 2014. Urbanization increases *Aedes albopictus* larval habitats and accelerates mosquito development and survivorship. *PLOS Negl Trop Dis* 8:e3301.

Linthicum KJ, Kramer VL, Madon MB, Fujioka KK, the Surveillance-Control Team. 2003. Introduction and potential establishment of *Aedes albopictus* in California in 2001. *J Am Mosq Control Assoc* 19:301–308.

Madon MB, Hazelrigg JE, Shaw MW, Kluh S, Mulla MS. 2003. Has *Aedes albopictus* established in California? *J Am Mosq Control Assoc* 19:297–300.

Mogi M. 1982. Variation in oviposition, hatch rate and setal morphology in laboratory strains of *Aedes albopictus*. *Mosq News* 42:196–201.

Moore CG. 1999. *Aedes albopictus* in the United States: current status and prospectus for further spread. *J Am Mosq Control Assoc* 15:221–227.

Moore CG, Fancy DB, Eliason DA, Monath TP. 1988. *Aedes albopictus* in the United States: rapid spread of a potential disease vector. *J Am Mosq Control Assoc* 4:356–361.

Peacock BE, Smith JP, Gregory PG, Loyless TM, Mulrennan JA Jr, Simmonds PR, Padgett L Jr, Cook EK, Eddins TR. 1988. *Aedes albopictus* in Florida. *J Am Mosq Control Assoc* 4:362–365.

Toma L, Severini F, Di Luca M, Bella A, Romi R. 2003. Seasonal patterns of oviposition and egg hatching rate of *Aedes albopictus* in Rome. *J Am Mosq Control Assoc* 19:19–22.

Urbanski JM, Benoit JB, Michaud MR, Denlinger DL, Armbruster P. 2010. The molecular physiology of increased egg desication resistance during diapause in the invasive mosquito, *Aedes albopictus*. *Proc Biol Sci* 277 (1694):2683–2692.

Vitek CJ, Livdahl TP. 2006. Field and laboratory comparison of hatch rates in *Aedes albopictus* (Skuse). *J Am Mosq Control Assoc* 22:609–614.

Wekesa JW, Sorvillo TE, Brisco A, Tanaka M, Cook M, Fujioka K. 2014. Three years (2011–2013) of *Aedes albopictus* in the City of El Monte, San Gabriel Valley, Los Angeles County, California. *Proc Papers Mosq Vector Control Assoc* 82:82–85.

Zhong D, Lo E, Hu R, Metzger ME, Cummings R, Bonnizzoni M, Fujioka KK, Sorvillo TE, Kluh S, Healy SP, Fredregill C, Kramer VL, Chen X, Yan G. 2013. Genetic analysis of invasive *Aedes albopictus* populations in Los Angeles County, California and its potential public health impact. *PLoS ONE* 8(7):e68586. https://doi.org/10.1371/journal.pone.0068586