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AGE-DEPENDENT BLOODFEEDING OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* ON ARTIFICIAL AND LIVING HOSTS

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ABSTRACT. In order to quantify age-dependent acquisition of a bloodmeal, we compared bloodfeeding patterns of *Aedes aegypti* and *Aedes albopictus* starting from the time of first responsiveness to a bloodmeal up to 15 days postemergence. In separate experiments, cohorts of *Ae. aegypti* and *Ae. albopictus* of known age were offered their first bloodmeal from a silicon-membrane system or a restrained chicken. For cohorts ranging from 3 to 15 days old, the proportions feeding were significantly affected by species, age, feeding protocol, and the age × feeding protocol interaction. For both feeding protocols, a higher proportion of *Ae. aegypti* than *Ae. albopictus,* on average, consumed blood. Regressions of proportion feeding versus age indicated significantly positive slopes for *Ae. albopictus* and *Ae. aegypti* on the membrane system, and no significant relationship was observed for either species on the restrained chicken. Additionally, slopes for *Aedes,* as a group, fed on the membrane system were significantly different from those fed on the living host. For both *Aedes* species fed on the restrained chicken and for *Ae. aegypti* fed on the membrane system, the proportions feeding exhibited periodic patterns, with peaks approximately 2 days apart, suggesting possible control by endogenous rhythms.

KEY WORDS Female age, bloodfeeding, periodic patterns, *Aedes aegypti, Aedes albopictus*

INTRODUCTION

Since its introduction to the Americas in the mid-1980s (Sprenger and Wuithiranyagool 1986, Hawley et al. 1987), *Aedes albopictus* (Skuse) has spread rapidly and colonized much of the southeastern USA and Brazil. In parts of eastern USA, the invasion of *Ae. albopictus* is associated with declines in the abundance, and in some instances displacement, of *Aedes aegypti* (L.) in rural and suburban areas (Hobbs et al. 1991, Hornby et al. 1994, Mekuria and Hyatt 1995, O'Meara et al. 1995). However, these *Aedes* coexist in urban areas of south Florida. Recent comparative studies attempting to explain the observed distributions of these *Aedes* have investigated egg desiccation (Sota and Mogi 1992, Juliano et al. 2002), larval competition (Barrera 1996, Juliano 1998, Daugherty et al. 2000, Lounibos et al. 2002), adult desiccation (Mogi et al. 1996), and reproductive and metabolic differences (Klowden and Chambers 1992).

One major concern about the *Ae. albopictus* invasion in the Americas has been its potential as an arboviral disease vector (e.g., dengue). In recent decades, the range of *Ae. aegypti,* the primary vector of dengue in the Americas, has increased, and dengue activity has surged (Gubler 1997). The range of *Ae. albopictus* in the USA is more extensive than that of *Ae. aegypti,* and its range in the USA is likely to continue to expand (e.g., Madon et al. 2002). *Aedes albopictus* is a competent laboratory vector of numerous arboviruses (Shroyer 1986, Mitchell 1991), including dengue in Asia and Hawaii; however, the degree to which *Ae. albopictus* is involved in arbovirus transmission in the Americas is unclear.

With exceptions of transovarial and venereal transmission, successful biological transmission of arboviruses requires acquisition of an infectious bloodmeal or at least probing behavior. For *Ae. aegypti,* research investigating factors that influence the normal sequence of events in successful acquisition of a bloodmeal (e.g., host-seeking, probing, bloodfeeding) have mainly focused on measurements of host-seeking behavior (Klowden and Lea 1978, 1979a, 1979b, 1984, Klowden et al. 1988; Bowen 1991, Klowden and Briegel 1994, Klowden and Fernandez 1996). Davis (1984) showed a linear increase in host-seeking behavior of *Ae. aegypti* from 1 to 5 days postemergence followed by a constant high response until the end of observations at 15 days. A study measuring probing behavior in *Ae. aegypti* over a 21-day period showed a rhythmic pattern in probing activity in response to a convection current of constant heat and moisture, but no probing pattern was observed in response to a human host (Burgess 1959). However, the design of this latter experiment was weak (e.g., experimental units were not replicated) and there was little statistical support for the conclusion of rhythmic behavior. Few studies have measured age-related acquisition of the initial bloodmeal, an important factor in determining vector potential; those that have done so have focused on bloodfeeding over a short interval. Seaton and Lumsden (1941) showed a general increase in bloodfeeding associated with age for 1–5-day-old starved virgin *Ae. aegypti* followed by decreased bloodfeeding on day 6. They suggested that the decreased response on day 6 was attributable to female exhaustion. A similar increase in bloodfeeding with increasing age was found for 3 strains of 1–4-day-old starved *Ae. aegypti* fed on chickens and membrane systems (Bishop and Gilchrist 1946). In order to quantify

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age-dependent acquisition of a bloodmeal, the present study compares bloodfeeding patterns of *Ae. albopictus* and *Ae. aegypti* starting from the time of first responsiveness to a bloodmeal (Christophers 1960, Hawley 1988) up to 15 days postemergence.

MATERIALS AND METHODS

Experimental Protocol

Aedes eggs used to initiate the experiments were derived from laboratory colonies at the Florida Medical Entomology Laboratory in Vero Beach, FL. Both *Aedes* spp. originated from Fall, 2000, field collections from water-filled cemetery vases in Hillsborough County, FL, near Tampa. Colonies were housed in 0.03-m^3 cages at (mean ± SD) 24.6 ± 0.4°C, 76.6 ± 6.7% relative humidity (RH), and a 14:10 (L:D)-h photoperiod regime including a 1-h dawn and dusk. Colonies had access to ≈20% sucrose solution ad libitum and weekly bloodmeals from domestic chickens (handled in accordance with the National Institutes of Health guidelines for the use of laboratory animals). Females were provided with water-containing cups lined with paper towel as oviposition substrates. Eggs were hatched, by species, in metal pans with 1.0 liter tap water and 0.30 g of a 1:1 lactalbumin and brewers yeast mixture. Following hatching, approximately 300–500 larvae were reared in pans, with water and food substrate changed every 2 days.

As soon as pupation occurred, inspections of the rearing pans were made daily, and we transferred pupae into 40-ml vials with water until emergence. Vials were checked daily between 1600 and 1800 h for newly emerged adults. These adults were transferred, by species, to cylindrical cages (11 × 9.5 cm, ht × diam) with nylon mesh tops and maintained under similar conditions as the parental generation except for bloodfeeding. Female density per cage ranged from 5 to 47 with means ± SE of 14.8 ± 9.9 and 14.2 ± 8.2 for *Ae. aegypti* and *Ae. albopictus,* respectively. At least one male was present in each cage for every 3–4 females, although many cages had equal numbers of males and females. Examination of scatter plots of residuals versus predicted values (Draper and Smith 1966) showed no evidence that the number of males per cage was in any way related to proportions of females that bloodfed.

In experiment 1, cages with *Aedes* females were haphazardly assigned to an age treatment (e.g., 3, 4, . . ., 15 days old). Each cage containing same-age adults ranging from 3 through 15 days old was offered a bloodmeal from a silicon-membrane feeding system (Butler et al. 1984). Thus, same-age females were tested on many different days. Females were deprived of sucrose, but not water, 24 h prior to bloodfeeding trials. Before the start of a feeding trial, citrated bovine blood was heated to (mean ± SD) 37.8 ± 1.1°C in 1.5-ml circular wells and covered with a silicon membrane. Next, the membrane feeding system was positioned over the mesh top of the cage for two 15-min periods separated by a 15-min interval. Feeding trials were performed at (mean ± SD) 23.2 ± 0.5°C and 48.1 ± 4.2% RH. After a feeding trial, the number of females that successfully acquired a bloodmeal was recorded. If blood was visually detected in the female gut, it was scored as having a bloodmeal. Thus, no attempt was made to distinguish between meals of different volumes. All feeding trials were performed in the late afternoon, within 2–3 h of each other.

For experiment 2, 3–15-day-old *Aedes* were allowed to bloodfeed from a restrained domestic chicken. The methods for mosquito husbandry and adult exposure during feeding trials were the same as those used in experiment 1. For all feeding trials, uniformly sized and aged (6–8-wk-old) chickens were restrained inside 0.03-m^3 cages into which adult *Aedes* were released and allowed to feed for 30 min. Female density per cage ranged from 5 to 51, with means ± SE of 28.1 ± 9.9 and 24.1 ± 9.7, for *Ae. aegypti* and *Ae. albopictus,* respectively. We used larger cages for bloodfeeding in experiment 2 to provide greater space for the normal sequence of events involved in bloodmeal acquisition (Clements 1999). Immediately following feeding trials, chickens were removed from the cages, and then *Aedes* were removed from the cage using an electric aspirator and killed by placing them at −20°C for <1 h. The number of female *Aedes* that had successfully bloodfed was recorded as in experiment 1.

Data Analyses

For experiments 1 and 2, proportions bloodfed for each species were calculated as the numbers of females that acquired a bloodmeal during a trial divided by the total numbers of females offered the bloodmeal. We defined our experimental unit as the cage of adult *Aedes* offered blood. Difficulties in predicting the number of females that would emerge and survive to the day of feeding precluded equal sample sizes for each unique species-by-age treatment. For experiment 1, the numbers of replicates for each *Ae. aegypti*- and *Ae. albopictus*-by-age treatment were (mean ± SD) 5 ± 1 and 5 ± 2, respectively (127 total cages). In experiment 2, we restricted each unique species-by-age treatment to 3 replicates, except for 15-day-old *Ae. aegypti,* which had 4 replicates (79 total cages).

For both experiments, we tested for effects of female density per cage as a continuous variable (SAS Institute 1989, PROC GLM; Sokal and Rohlf 1995). Raw data adequately met assumptions of normality and homogeneous variance except for membrane-fed *Ae. albopictus,* where the proportion bloodfed was transformed by $\log_{10}(x + 1)$ to meet the assumption of normality. Because effects of fe-

Table 1. Test for equal slopes among regressions of proportion bloodfed of *Aedes aegypti* and *Aedes albopictus* versus age.

Source	df	Type III SS	MS	F	*P*
Age	1	1.3739	1.3739	30.69	<0.0001
Feeding protocol	1	1.4164	1.4164	31.64	<0.0001
Species	1	0.1824	0.1824	4.07	0.0449
Feeding protocol × species	1	0.0697	0.0697	1.56	0.2136
Age × feeding protocol	1	0.2671	0.2671	5.97	0.0155
Age × species	1	0.0412	0.0412	0.92	0.3384
Age × feeding protocol × species	1	0.1094	0.1094	2.44	0.1197
Error df	198				

male density on proportion bloodfed were all nonsignificant ($P \gg 0.10$ in all cases), we proceeded with analysis of effects of female age on proportion bloodfed, which was assessed by treating age as a continuous independent variable and comparing regression lines for each species by feeding protocol treatment. This tests for equal slopes among species-feeding protocol groups to determine whether the regression relationships were similar (SAS Institute 1989, Sokal and Rohlf 1995).

Graphical presentation of our data appeared to show age-dependent periodicity in feeding incidence. Therefore, we ran separate Runs Up and Down Tests for the proportion bloodfed for each species-feeding protocol combination (Sokal and Rohlf 1995, Zar 1996). We defined a run as a temporal sequence of increases or decreases in the proportion bloodfed. We determined the difference between mean proportion bloodfed for consecutive age groups, resulting in a sequence of positive and negative changes in proportion bloodfed across female ages (e.g., + + + − − = 2 runs). These tests enabled us to determine whether the number of runs for proportion bloodfed among females of different ages was significantly different from random expectation.

As an additional test to address the apparent age-dependent periodic pattern of feeding, we ran 4 regressions (1 for each species-feeding protocol combination) of proportion bloodfed versus age, each with a sine function of age according to the model:

$$y = a + bA + c \sin(dA), \qquad [1]$$

where y is the proportion bloodfed, a is the intercept, b is the slope, A is female age, c is a parameter affecting the amplitude of the sine function, and d is a parameter affecting the frequency of the sine function. Several different initial parameter estimates were used to determine whether the addition of a sine wave function improved the fit of the regression (SAS Institute 1989, PROC NLIN). If either c or d parameters were not significant, the slope (b) was removed and the reduced model tested. Subsequently, if either c or d were not significant, both c and d were removed from the model, and we ran a linear regression, including the slope, to determine whether or not there was a trend in age-dependent bloodfeeding.

RESULTS

Regardless of age, a higher proportion of both *Ae. albopictus* and *Ae. aegypti* bloodfed on the restrained chicken (mean ± SE; 59.8 ± 2.4 and 81.3 ± 2.3%, respectively) compared with the membrane system (mean ± SE; 30.8 ± 2.7 and 55.6 ± 2.6%, respectively).

Treating age as a continuous independent variable, there were significant age, species, feeding protocol, and age × feeding protocol effects (Table 1). All other effects were not significant. Slopes of proportion bloodfed versus age were significantly positive for both *Aedes* species feeding on the membrane system and were not significantly different from zero for both *Aedes* species feeding on the restrained chicken (Table 2). Although these slopes were significant, the low r^2 values suggest that the linear relationships were weak (Fig. 1, Table 2). In addition, the sine function contributed significantly to the regression for *Ae. aegypti* ($P < 0.0001$, $r^2 = 0.391$, proportion fed = 0.188 + 0.042 × (age) + 0.11 sine(9.91 × age)). For both *Aedes* species fed on the restrained chicken, the sine function did not contribute significantly to the regression (Table 2). Averaged over both species, slopes for proportion bloodfed on the membrane system

Table 2. Intercept and slope estimates for simple linear regressions of proportion bloodfed of *Aedes aegypti* and *Aedes albopictus* versus age. Slopes for groups followed by different letters are significantly different.

Source		Intercept ± SE	Slope ± SE		r^2	df	F	*P*
Membrane System	*Ae. aegypti*	0.1703 ± 0.0779	0.0434 ± 0.0082	a	0.3171	1,61	28.33	<0.0001
	Ae. albopictus	0.1084 ± 0.0778	0.0226 ± 0.0081		0.1110	1,62	7.74	0.0071
Chicken host	*Ae. aegypti*	0.7225 ± 0.0593	0.0103 ± 0.0059	b	0.0723	1,38	2.96	0.0934
	Ae. albopictus	0.4600 ± 0.0879	0.0153 ± 0.0090		0.0720	1,37	2.87	0.0987

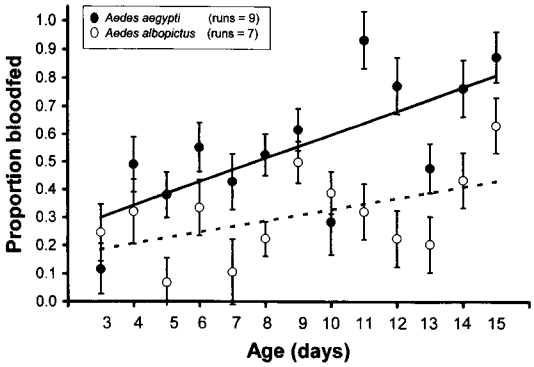

Fig. 1. Least squares means (± SE) for proportion bloodfed females on the silicon-membrane system for 3–15-day-old *Aedes albopictus* and *Ae. aegypti.* Line drawn through means shows the best-fit linear regressions for *Ae. aegypti* (solid) and *Ae. albopictus* (broken).

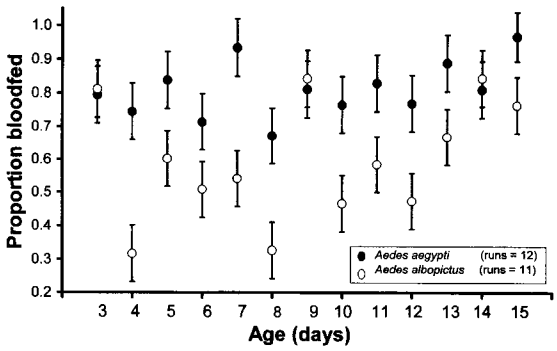

Fig. 2. Least squares means (± SE) for proportion bloodfed females on restrained chickens for 3–15-day-old *Aedes albopictus* and *Ae. aegypti.*

were significantly greater, as shown by the age × feeding protocol interaction, than those for *Aedes* fed on the restrained chicken (Tables 1 and 2).

Runs Up and Down Tests for *Ae. albopictus* and *Ae. aegypti* fed on restrained chickens showed that the number of runs was significantly different from random (both $P < 0.0275$), with the number of runs being greater than expected compared with random (Fig. 2). Thus, proportion bloodfed on chickens showed a significant pattern of alternate increases and decreases on alternate days of female mosquito age. Runs Up and Down Tests were not significant (both $P > 0.05$) for either *Aedes* species fed on the membrane system (Fig. 1).

DISCUSSION

In experiment 1, using the membrane feeders, there was a significant increase in proportion bloodfed as age increased (Table 2, Fig. 1). In experiment 2, with restrained chickens, there was no significant increase in bloodfeeding associated with increased age (Table 2, Fig. 2). Further, the membrane-fed and chicken-fed mosquitoes showed significantly different trends (Table 2). Thus, the temporal pattern of bloodfeeding is strongly affected by the blood source used in experiments. Host-related cues (e.g., CO_2) may be partially responsible for the observed differences in pattern of bloodfeeding and should be taken into consideration in bloodfeeding research using *Aedes* mosquitoes of different ages, especially for silicon-membrane systems. The lack of significantly positive slopes for chicken-fed mosquitoes is likely due to a higher proportion of bloodfed younger *Aedes* as compared with the membrane-fed mosquitoes.

Our results show significant age effects on bloodfeeding for both *Ae. aegypti* and *Ae. albopictus.* Davis (1984), in a study with naive *Ae. aegypti* females of uniform ages ranging from 1 through 15 days old, showed a linear increase in host-seeking behavior for 1–5-day-old females, whereas females >5 days old showed a consistently high (e.g., ≈94%) response to a human hand. Results from the current study suggest that bloodfeeding for these *Aedes,* over a similar period of time, shows some similarities to host-seeking response observed by Davis. However, proportions bloodfeeding appear additionally to exhibit distinct periodic patterns on alternate days of female mosquito age. We found significant but weak positive relationships for *Ae. albopictus* and *Ae. aegypti* feeding versus age on the membrane system (Fig. 1, Table 2) and no positive relationships for feeding versus age on the restrained chicken (Fig. 2, Table 2). Additionally, slopes for the 2 *Aedes* species, as a single group, fed on the membrane system were significantly different from those fed on the restrained chicken (Table 2). Also, there appears to be an age-dependent periodic pattern in bloodfeeding incidences. The periodic pattern is most obvious among 3–13-day-old adults of each species fed on the restrained chicken, where the number of runs was significantly greater than that expected for random daily variation. Likewise, for the proportion of bloodfed *Ae. aegypti* on the membrane system versus age, a sine function made a significant contribution to the fit, providing further evidence for periodicity. This result is surprising because this was a short time series and typically, time series analyses have the potential to provide good fits when there are >50 observations (Chatfield 1989). Unlike some previous research, *Aedes* in the current study were experimentally naive (i.e., never given a previous bloodmeal); thus, any periodicity in the time series is likely attributable to endogenous factors. Periodicity could be an artifact of unknown exogenous factors, although we attempted to control these factors (e.g., temperature, humidity, feeding times). These results lend support to previous reports of a possible periodic pattern in probing behavior of nonbloodfed *Ae. aegypti* (Burgess 1959) and host-seeking behavior in nonbloodfed *Anopheles gambiae* sensu stricto (Takken et al. 1998).

Hormone levels (e.g., juvenile hormone, ecdysteroids) vary at different times throughout the duration of adult female life. Juvenile hormone has been shown to be involved in initiating bloodfeeding for *Culex pipiens* (L.) and *Cx. quinquefasciatus* (Say) (Meola and Petralia 1980), and *Cx. nigripalpus* (Theobald) (Hancock and Foster 2000). The processes by which synergistic and antagonistic effects of juvenile hormone and ecdysteriods, from day to day, influence consumption of the initial bloodmeal, especially long after emergence (e.g., 15 days), is unknown. Given the lack of data on endogenous hormone fluctuation during the life span of unfed females, we can only speculate that these hormones may contribute to the apparent age-dependent differences observed in proportion bloodfed of *Ae. aegypti* and *Ae. albopictus.*

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REFERENCES CITED

Barrera R. 1996. Competition and resistance to starvation in container-inhabiting *Aedes* mosquitoes. *Ecol Entomol* 21:117–127.

Bishop A, Gilchrist BM. 1946. Experiments upon the feeding of *Aedes aegypti* through animal membranes with a view to applying this method to the chemotherapy of malaria. *Parasitology* 37:85–100.

Bowen MF. 1991. The sensory physiology of host-seeking behavior in mosquitoes. *Annu Rev Entomol* 36:139–158.

Burgess L. 1959. Probing behaviour of *Aedes aegypti* (L.) in response to heat and moisture. *Nature* 184:1968–1969.

Butler JF, Hess WR, Endris RG, Holscher KH. 1984. *In vitro* feeding of *Ornithodoros* ticks for rearing and assessment of disease transmission. *Acarology* VI:1075–1081.

Chatfield C. 1989. *The analysis of time series. An introduction* New York: Chapman and Hall.

Christophers SR. 1960. Aedes aegypti *(L.) The yellow fever mosquito* London: Cambridge University Press.

Clements AN. 1999. *The biology of mosquitoes,* Volume 2, *Sensory reception and behaviour* New York: CABI Publishing.

Daugherty MP, Alto BW, Juliano SA. 2000. Invertebrate carcasses as a resource for competing *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 37:364–372.

Davis EE. 1984. Development of lactic acid-receptor sensitivity and host-seeking behaviour in newly emerged female *Aedes aegypti* mosquitoes. *J Insect Physiol* 30: 211–215.

Draper NR, Smith H. 1966. *Applied regression analysis* New York: Wiley.

Gubler DJ. 1997. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: Gubler DJ, Kuno G, eds. *Dengue and dengue hemorrhagic fever* New York: CABI Publishing. p 1–22.

Hancock RG, Foster WA. 2000. Exogenous juvenile hormone and methoprene, but not male accessory gland substances or ovariectomy, affect the blood/nectar choice of female *Culex nigripalpus* mosquitoes. *Med Vet Entomol* 14:376–382.

Hawley WA. 1988. The biology of *Aedes albopictus. J Am Mosq Control Assoc* 1(Suppl):1–40.

Hawley WA, Reiter P, Copeland RS, Pumpuni CB, Craig GB Jr. 1987. *Aedes albopictus* in North America: probable introduction in used tires from Northern Asia. *Science* 236:1114–1116.

Hobbs JH, Hughes EA, Eichold BH, II. 1991. Replacement of *Aedes aegypti* by *Aedes albopictus* in Mobile Alabama. *J Am Mosq Control Assoc* 7:488–489.

Hornby JA, Moore DE, Miller TW Jr. 1994. *Aedes albopictus* distribution, abundance, and colonization in Lee County, Florida, and its effect on *Aedes aegypti. J Am Mosq Control Assoc* 10:397–402.

Juliano SA. 1998. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition. *Ecology* 79:255–268.

Juliano SA, O'Meara GF, Morrill JR, Cutwa MM. 2002. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. *Oecologia (Heidelb)* 130:458–469.

Klowden MJ, Lea AO. 1978. Blood meal size as a factor affecting continued host-seeking by *Aedes aegypti* (L.). *Am J Trop Med Hyg* 27:827–831.

Klowden MJ, Lea AO. 1979a. Humoral inhibition of host-seeking in *Aedes aegypti* during oocyte maturation. *J Insect Physiol* 25:231–235.

Klowden MJ, Lea AO. 1979b. Abdominal distention terminates subsequent host-seeking behaviour of *Aedes aegypti* following a blood meal. *J Insect Physiol* 25: 583–585.

Klowden MJ, Lea AO. 1984. Blood feeding affects age-related changes in the host-seeking behavior of *Aedes aegypti* (Diptera: Culicidae) during oocyte maturation. *J Med Entomol* 21:274–277.

Klowden MJ, Blackmer JL, Chambers GM. 1988. Effects of larval nutrition on the host-seeking behavior of adult *Aedes aegypti* mosquitoes. *J Am Mosq Control Assoc* 4:73–75.

Klowden MJ, Briegel H. 1994. Mosquito gonotrophic cycle and multiple feeding potential: contrasts between *Anopheles* and *Aedes. J Med Entomol* 31:618–622.

Klowden MJ, Chambers GM. 1992. Reproductive and metabolic differences between *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae). *J Med Entomol* 29: 467–471.

Klowden MJ, Fernandez NM. 1996. Effects of age and mating on the host seeking behavior of *Aedes aegypti* mosquitoes. *J Vector Ecol* 21:156–158.

Lounibos LP, Suarez S, Menendez Z, Nishimura N, Escher RL, O'Connell SM, Rey JR. 2002. Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*? *J Vector Ecol* 27:86–95.

Madon MB, Mulla MS, Shaw MW, Kluh S, Hazelrigg JE. 2002. Introduction of *Aedes albopictus* (Skuse) into Southern California and potential for its establishment. *J Vector Ecol* 27:149–154.

Mekuria Y, Hyatt MG. 1995. *Aedes albopictus* in South Carolina. *J Am Mosq Control Assoc* 11:468–470.

Meola RW, Petralia RS. 1980. Juvenile hormone induction of biting behavior in *Culex* mosquitoes. *Science* 209: 1548–1550.

Mitchell CJ. 1991. Vector competence of North and South American strains of *Aedes albopictus* for certain arboviruses: a review. *J Am Mosq Control Assoc* 7:446–451.

Mogi M, Miyagi I, Abadi K, Syafruddin. 1996. Inter- and intraspecific variation in resistance to desiccation by adult *Aedes* (*Stegomyia*) spp. (Diptera: Culicidae) from Indonesia. *J Med Entomol* 33:53–57.

O'Meara GF, Evans LF, Gettman AD Jr, Cuda JP. 1995. Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera: Culicidae) in Florida. *J Med Entomol* 32:554–562.

SAS Institute, Inc. 1989. *SAS/STAT® users' guide version 6.* Cary, NC: SAS Institute, Inc.

Seaton DR, Lumsden WHR. 1941. Observations on the effects of age, fertilization and light on biting by *Aedes aegypti* (L.) in a controlled microclimate. *Ann Trop Med Parasitol* 35:23–36.

Shroyer DA. 1986. *Aedes albopictus* and arboviruses: a concise review of the literature. *J Am Mosq Control Assoc* 2:424–428.

Sokal RR, Rohlf FJ. 1995. *Biometry.* New York: W.H. Freeman and Company.

Sota T, Mogi M. 1992. Interspecific variation in desiccation survival time of *Aedes* (*Stegomyia*) mosquito eggs is correlated with habitat and egg size. *Oecologia (Heidelb)* 90:353–358.

Sprenger D, Wuithiranyagool T. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J Am Mosq Control Assoc* 2:217–219.

Takken W, Klowden MJ, Chambers GM. 1998. Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae): the disadvantage of being small. *J Med Entomol* 35: 639–645.

Zar JH. 1996. *Biostatistical analysis* New Jersey: Prentice Hall.