MATING AND NUTRITIONAL STATE AFFECT THE REPRODUCTION OF *AEDES ALBOPICTUS* MOSQUITOES

MARC J. KLOWDEN

Division of Entomology, University of Idaho, Moscow, ID 83844

ABSTRACT. Mated female *Aedes albopictus* mosquitoes that were maintained after emergence without carbohydrate were more likely to develop eggs after a small blood meal than were unmated females maintained on the same diet. The effect was due to male accessory gland substances transferred to the female during mating. Neither the endogenous reserves of protein and lipid nor the number of eggs developed per volume of ingested blood differed between mated and unmated females, suggesting that the utilization of existing reserves was altered by mating. Methoprene administered to both mated and unmated females that ingested small blood meals reduced the likelihood that egg development would occur. Small volumes of blood were more likely to trigger oogenesis in both mated and unmated females if their abdomens were additionally distended.

INTRODUCTION

The mosquitoes *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse) are related species that occupy similar ecological niches. The distribution of both species has increased as a result of their ability to colonize containers in and around human habitats. When their distributions have overlapped, one of the 2 species has generally been supplanted (Gilotra et al. 1967, Mogi et al. 1988, Hobbs et al. 1991), but the ecological circumstances under which one species might be replaced by another are difficult to predict. The competitive interactions between the larvae of these species have most often been used to explain their distribution patterns (Macdonald 1956, Moore and Fisher 1969, Black et al. 1989).

When maintained on carbohydrate-restricted diets, *Aedes albopictus* is more likely to produce eggs after ingesting small blood meals than is *Aedes aegypti* (Klowden and Chambers 1992). Although some of this difference may be due to the longer larval period of *Ae. albopictus* that allows it to accumulate greater lipid and glycogen reserves, it appears that the adult reproductive metabolism of the two species is different, a difference that may partially explain the outcome of competitive interactions. In this study, the reproductive metabolism of *Ae. albopictus* was further explored, with an examination of the relationship between nutritional state and the male accessory gland substances that are contributed during mating.

MATERIALS AND METHODS

The *Ae. albopictus* colony was obtained from individuals originally colonized in Lake Charles, Louisiana. Larvae were reared at 27°C and fed daily until pupation with increasing amounts of powdered rat chow-lactalbumin hydrolysate-brewers yeast (1:1:1 by weight). Both larvae and adults were raised under a 14:10 (L:D) photoperiod, and starved adults were maintained at 80% RH with access to only water from cotton wicks. Sugar-fed adults were fed a 10% sucrose solution. Approximately 10% of the stock population developed a first batch of eggs autogenously, but only when the females were provided with sucrose after emergence. Autogeny was never observed in the experimental groups that did not ingest carbohydrate.

Accessory reproductive glands were removed from 4-day-old unmated, sucrose-fed males and implanted into the abdomens of unmated females through a slit in the arthrodial membrane. The wounds were sealed with molten paraffin. In controls, the arthrodial membrane was opened and immediately sealed. Adult females were given measured amounts of heparinized rat blood administered as enemas (Briegel and Lea 1975). They were dissected and examined for eggs 2 days later.

Nutritional reserves of carbohydrate and lipid in individual mosquitoes were assessed by the methods of Van Handel (1985a, 1985b) performed as described previously (Klowden and Chambers 1992). Total protein in mated and unmated females was determined by first sonicating individual mosquitoes in 0.01 N NaOH, centrifuging to pellet insoluble portions, and assaying an aliquot of the supernatant by the method of Bradford (1976) based on a standard curve of bovine serum albumin.

Statistical differences between the responses of experimental groups were determined by transforming percentages to their arcsine values and testing for their equality (Sokal and Rohlf 1969). Regression lines were compared by the method of Gomez and Gomez (1984).

RESULTS

More than 90% of the mated *Ae. albopictus* females that were maintained on water for 4 days after emergence and then fed 1 μl or more

of blood developed eggs. Oogenesis was initiated in about 20% of the mated population even when as little as 0.1 μl of blood was ingested (Fig. 1). However, unmated females had a much higher threshold for oogenesis, requiring 0.25 μl or more of blood before eggs were produced. Increasing volumes of blood up to 1 μl initiated oogenesis in an increasing proportion of the unmated population, but significantly fewer developed eggs compared with mated females given the same volume of blood (Fig. 1). Although there appeared to be significant differences in the response of mated and unmated females to blood ingestion, the relationship between the number of eggs produced and the volume of blood ingested did not differ significantly between mated and unmated females (Fig. 2).

To determine whether the difference in their response to blood ingestion may have resulted from differences in their nutritional reserves, mated and unmated females were analyzed for total glycogen, lipid and protein. Glycogen content was significantly greater in unmated females, but the low levels found in both mated and unmated females were not biologically meaningful. In contrast, there were no significant differences between the much larger lipid and protein reserves in mated and unmated females (Table 1).

Because it appeared that mating affected egg development, single accessory glands from male *Ae. albopictus* were implanted into unmated *Ae. albopictus* females, which were then given 0.5 μl of blood by enema. As shown in Table 2, the implantation of the glands significantly increased the ability of the unmated females to

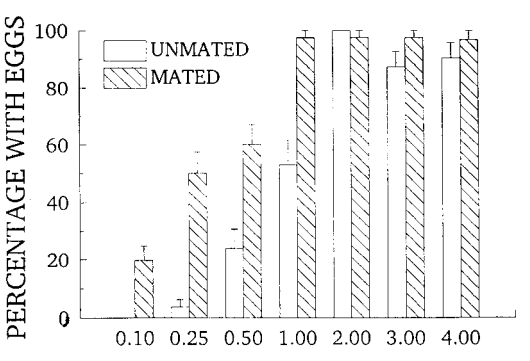

Fig. 1. Effect of increasing blood meal size on the percentage of mated and unmated *Aedes albopictus* that developed eggs. The percentages for mated and unmated females differ significantly ($P < 0.05$) with blood volumes of 1 μl and below. Vertical bars represent standard errors.

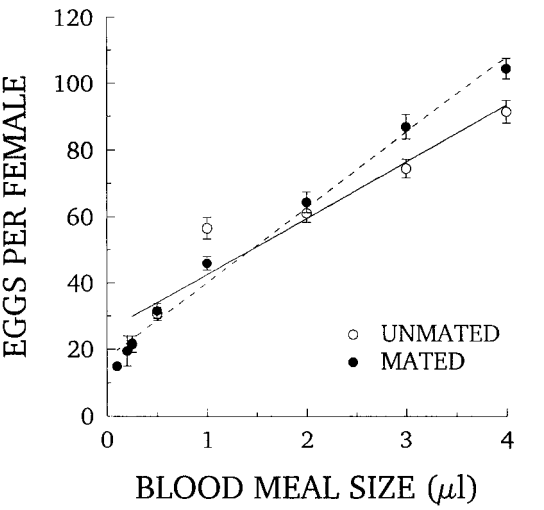

Fig. 2. Effect of increasing blood meal size on the number of eggs developing per female in mated and unmated *Aedes albopictus*. The regression lines for mated ($Y = 17.6 + 22.5X$; $r^2 = 0.98$) and unmated ($Y = 25.6 + 16.9X$; $r^2 = 0.92$) females did not differ significantly ($t = 2.04$; $P > 0.05$). Vertical bars represent standard errors.

Table 1. Whole body glycogen, lipid, and protein of mated and unmated *Aedes albopictus* females maintained on water for 4 days following emergence.

	Micrograms/female ± SE		
Treatment	Glycogen	Lipid	Protein
Mated	5.5 ± 0.7[a]*	166.7 ± 5.7[a]	254.1 ± 2.7[a]
Unmated	8.5 ± 1.0[b]	174.4 ± 6.2[a]	255.9 ± 2.4[a]

* Values followed by the same letter within a column are not significantly different ($P > 0.05$); $n = 60$ for all treatments.

Table 2. Effect of whole male accessory gland (MAG) implants from *Aedes albopictus* or *Ae. aegypti* into unmated *Ae. albopictus* females. Following implantation, the hosts were given 0.5 μl of blood.

Treatment	Percentage with eggs (±SE)	*n*
Sham-operated controls	22.7 ± 6.3	41
MAG from *Ae. albopictus*	47.6 ± 10.9*	21
MAG from *Ae. aegypti*	13.3 ± 8.8	25

* Indicates a significant difference from controls ($P < 0.05$).

develop eggs. However, unmated *Ae. albopictus* implanted with a male accessory gland from *Ae. aegypti* were not affected, and their response to blood did not differ significantly from sham-operated controls.

Abdominal distention provides nervous signals that affect oogenesis differently in mated and unmated *Ae. aegypti* (Klowden and Chambers 1991). Mated and unmated *Ae. albopictus* were given either their threshold blood volumes for oogenesis (0.1 μl for mated and 0.25 μl for unmated), or an additional volume of saline along with the blood to provide a total meal volume of 1 μl. The percentage with egg development was increased in both mated and unmated females by the additional abdominal distention from the extra saline in the meal (Table 3).

To determine whether the juvenile hormone analog methoprene affected egg development, either 1.0 or 0.1 μg of methoprene was applied to the abdomens of mated and unmated mosquitoes shortly after they were given 1 μl of blood. As shown in Fig. 3, topical application of methoprene reduced the proportion of both mated and unmated *Ae. albopictus* that developed eggs from the blood.

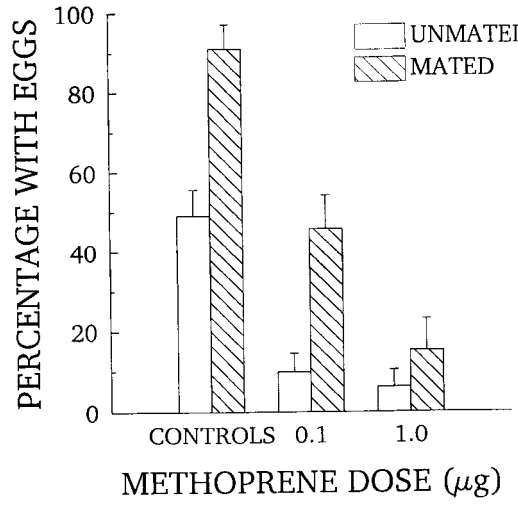

Fig. 3. Effects of topically applied methoprene on mated and unmated *Aedes albopictus* females that were fed 1 μl of blood. Controls received the same amount of blood but were treated with acetone only. Vertical bars represent standard errors.

DISCUSSION

Components of the male accessory reproductive gland affect the reproductive metabolism of starved *Ae. albopictus* females, allowing them to redirect resources and develop eggs that would otherwise not be produced (Fig. 1). These accessory gland components do not appear to act by affecting the pool of metabolic reserves, because although glycogen levels were actually greater in unmated females, perhaps because males caused the mated females to expend more energy for flight, protein and lipid levels were not significantly different (Table 1). Also, the numbers of eggs developed per unit of blood ingested did not differ between mated and unmated females (Fig. 2). However, mating or accessory gland implantation increased the probability that eggs would develop from a small blood meal (Fig. 1, Table 2). Therefore, a mechanism of male-induced oogenesis similar to that previously described for *Ae. aegypti* (Klowden and Chambers 1991) appears to also be present in *Ae. albopictus*.

It is generally believed that male-derived substances in insects are broken down and directly utilized or incorporated into the eggs of the female (Young and Downe 1987, Boggs 1990). However, the present data suggested that in mosquitoes, male accessory glands specifically affected the reproductive metabolism of the female and directed the manner in which existing reserves were utilized. Glands from *Ae. aegypti*, which were unlikely to differ significantly from those of *Ae. albopictus* in the total raw materials they contained, did not stimulate this male-induced oogenesis in *Ae. albopictus* (Table 2), although glands of *Ae. albopictus* are active in

Table 3. Effect of abdominal distention on oogenesis in mated and unmated *Aedes albopictus* receiving equivalent volumes of blood.

	Percentage with eggs (±SE)				
Treatment	Blood alone*	*n*	Blood and saline*	*n*	Significance
Mated females	19.7 ± 3.5	61	46.4 ± 9.4	28	$P < 0.05$
Unmated females	5.1 ± 2.5	55	33.3 ± 8.2	33	$P < 0.05$

* Mated females received 0.1 μl of blood or 0.1 μl of blood plus 0.9 μl of saline. Unmated females received 0.25 μl of blood or 0.25 μl of blood plus 0.75 μl of saline.

Ae. aegypti (Klowden and Chambers 1991). A similar result was obtained by Leahy and Craig (1965) who, in their study of male-induced oviposition, found an increase in egg deposition in unmated *Ae. albopictus* females implanted with *Ae. albopictus* male accessory gland, but not with one from *Ae. aegypti*. Thus, although the signal from the *albopictus* gland is recognized by *aegypti*, the reverse is not true.

Because exogenous juvenile hormone and methoprene appear to mobilize nutritional reserves in *Ae. aegypti* (Klowden and Chambers 1989), and juvenile hormone is a component in the secretions of some insect male accessory glands (Shirk et al. 1976, 1980), we speculated that juvenile hormone may be transferred by male accessory gland secretions in mosquitoes. However, contrary to our previous results with *Ae. aegypti* where it was observed that juvenile hormone elevated the percentage of unmated females that developed eggs (Klowden and Chambers 1991), exogenously applied methoprene inhibited oogenesis in *Ae. albopictus* (Fig. 3). If juvenile hormone is transferred to the female in male accessory gland components and is responsible for the increase in the ability of mated *Ae. albopictus* to develop eggs, topical doses of methoprene should not have the opposite effect. Also, the specificity of the transplanted glands argues against an induction by juvenile hormone, especially because *Ae. albopictus* glands are effective in *Ae. aegypti* (Leahy and Craig 1965, Klowden and Chambers 1991). These data do not support the hypothesis that juvenile hormone transferred in male accessory gland secretions affected the metabolic priorities in the nutritionally stressed mosquito.

Another difference between *Ae. aegypti* and *Ae. albopictus* is the interaction between mating and abdominal distention. When blood meal volume is kept constant and abdominal distention was increased, the percentage of *Ae. aegypti* that develop eggs increases only in mated females (Klowden and Chambers 1991). In contrast, both mated and unmated *Ae. albopictus* showed significant increases in the percentage that developed eggs when their abdomens were distended (Table 3). The mechanism by which abdominal distention stimulates mosquito oogenesis is not known, but may represent a second stimulus that acts along with humoral factors from the blood and initiates oogenesis when the quality of host blood is suboptimal.

ACKNOWLEDGMENTS

I thank G. M. Chambers for her technical assistance and R. S. Nasci for the *Aedes albopictus* colony. This research was supported in part by grant AI-24453 from the National Institutes of Health and contribution number 92760 from the Idaho Agricultural Experiment Station.

REFERENCES CITED

Black, W. C. IV, K. S. Rai, B. J. Turco and D. C. Arroyo. 1989. Laboratory study of competition between United States strains of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 26:260–271.

Boggs, C. L. 1990. A general model of the role of male-donated nutrients in female insects' reproduction. Am. Nat. 136:598–617.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.

Briegel, H. and A. O. Lea. 1975. Relationship between protein and proteolytic activity in the midgut of mosquitoes. J. Insect Physiol. 21:1597–1604.

Gilotra, K., L. E. Rozeboom and N. C. Bhattacharya. 1967. Observations on possible competitive displacement between populations of *Aedes aegypti* Linnaeus and *Aedes albopictus* Skuse in Calcutta. Bull. W.H.O. 37:437–446.

Gomez, K. A. and A. A. Gomez. 1984. Statistical procedures for agricultural research, 2nd edition. Wiley, New York.

Hobbs, J. H., E. A. Hughes and B. H. Eichold II. 1991. Replacement of *Aedes aegypti* by *Aedes albopictus* in Mobile, Alabama. J. Am. Mosq. Control Assoc. 7:488–489.

Klowden, M. J. and G. M. Chambers. 1989. Ovarian development and adult mortality in *Aedes aegypti* treated with sucrose, juvenile hormone and methoprene. J. Insect Physiol. 35:513–517.

Klowden, M. J. and G. M. Chambers. 1991. Male accessory gland substances activate egg development in nutritionally stressed *Aedes aegypti* mosquitoes. J. Insect Physiol. 37:721–726.

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

Leahy, M. G. and G. B. Craig, Jr. 1965. Accessory gland substance as a stimulant for oviposition in *Aedes aegypti* and *Ae. albopictus*. Mosq. News 25:448–452.

Macdonald, W. W. 1956. *Aedes aegypti* in Malaya. II. Larval and adult biology. Ann. Trop. Med. Parasitol. 50:399–414.

Mogi, M., C. Khamboonruang, W. Choochote and P. Suwanpanit. 1988. Ovitrap surveys of dengue vector mosquitoes in Chiang Mai, northern Thailand: seasonal shifts in relative abundance of *Aedes albopictus* and *Ae. aegypti*. Med. Vet. Entomol. 2:319–324.

Moore, C. G. and B. R. Fisher. 1969. Competition in mosquitoes. Density and species ratio effects on growth, mortality, fecundity, and production of growth retardant. Ann. Entomol. Soc. Am. 62:1325–1331.

Shirk, P. D., K. H. Dahm and H. Röller. 1976. The accessory sex glands as the repository for juvenile hormone in male Cecropia moths. Z. Naturforsch. 31C:199–200.

Shirk, P. D., G. Bhaskaran and H. Röller. 1980. The transfer of juvenile hormone from male to female during mating in the Cecropia silkmoth. Experientia 36:682–683.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. Freeman, San Francisco, CA.

Van Handel, E. 1985a. Rapid determination of glycogen and sugars in mosquitoes. J. Am. Mosq. Control Assoc. 1:299–301.

Van Handel, E. 1985b. Rapid determination of total lipids in mosquitoes. J. Am. Mosq. Control Assoc. 1:302–304.

Young, A. D. M. and A. E. R. Downe. 1987. Male accessory gland substances and the control of sexual receptivity in female *Culex tarsalis*. Physiol. Entomol. 12:233–239.