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EFFECT OF FEMALE SIZE ON FECUNDITY AND SURVIVORSHIP OF *AEDES AEGYPTI* FED ONLY HUMAN BLOOD VERSUS HUMAN BLOOD PLUS SUGAR

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ABSTRACT. We determined whether size, an indirect measure of teneral energy reserves, modifies the fitness advantage (Σ survival × egg production/female/day) conferred to female *Aedes aegypti* (L.) maintained on human blood over cohorts fed human blood plus sugar. Different sized females were obtained by rearing them at different larval densities and with different amounts of food per larva. Each female in 4 treatment groups of 23 mosquitoes each was maintained in a separate cage. A 10% sucrose solution was provided *ad libitum* to mosquitoes in the sugar-plus-blood treatments and water to the blood only groups. Eggs deposited and survival were monitored daily for each mosquito until all had died. Within a size category, survival of mosquitoes in different treatments was not different and mosquitoes fed only human blood laid more eggs than those fed blood plus sugar. The numbers of eggs laid by small mosquitoes fed human blood alone and large mosquitoes fed human blood plus sugar were not different. Mosquitoes fed only human blood had higher net replacement and intrinsic rates of growth than similar sized mosquitoes fed blood plus sugar. Female *Ae. aegypti* fed only human blood, regardless of the variation in size that we studied and thus energy reserves at emergence, had a fitness advantage over those fed a diet that included sugar.

KEY WORDS Fitness, blood feeding, mosquito, teneral, size, *Aedes aegypti*

INTRODUCTION

The feeding behavior of *Aedes aegypti* (L.) is unique among mosquito species that have been studied in detail because, compared with males, females seldom feed on sugar (Edman et al. 1992, Van Handel et al. 1994, Costero et al. 1998a). Instead, they preferentially, and frequently in each gonotrophic cycle, imbibe human blood (Scott et al. 1993a, 1993b). Results from recent studies that used a life table approach (Scott et al. 1997, Costero et al. 1998b) demonstrated that females fed only human blood have a fitness advantage (net replacement rate and intrinsic rate of growth) over cohorts fed human blood plus sugar. Mosquitoes included in those laboratory studies emerged from pupae collected from natural breeding sites. The fitness advantage was detected among *Ae. aegypti* collected in different regions of the world (Thailand and Puerto Rico) and at different times of the year (rainy versus dry season in Puerto Rico), which coincided with times of relatively high versus low dengue virus transmission.

This finding is epidemiologically important because an increase in a vector's biting rate on humans can exponentially increase transmission of a vector-borne pathogen (Garrett-Jones and Shidrawi 1969, Dye 1992). In the case of *Ae. aegypti,* the pathogen of primary interest is dengue virus (Gubler 1988). An extension of the studies cited above is that the feeding behavior of *Ae. aegypti* confers a selective advantage for both the mosquito and dengue virus (Scott et al. 1997). Feeding behavior may be a primary reason why *Ae. aegypti* is the most important arthropod vector of viral diseases to humans.

The objective of this study was to examine *Ae. aegypti* feeding behavior in greater detail by determining whether the reproductive advantage associated with feeding solely on human blood is modified by variation in female size and, therefore, indirectly by teneral energy reserves. Within natural populations, *Ae. aegypti* vary in size (Nasci 1990), and differences in size are positively correlated with significant differences in nutrient reserves at emergence (Briegel 1990). We sought to determine whether differences in energy reserves early in adult life alter the advantages of feeding only on human blood.

MATERIALS AND METHODS

The *Ae. aegypti* colony, methods for rearing large and small mosquitoes, and conditions under which immature mosquitoes were held are described by Naksathit et al. (1998).

In order to allow them to mate, ~100 teneral male and female mosquitoes in each of the two size categories were placed in 2 cages (30 × 30 × 30 cm, total of 4 cages). Within a size category, mosquitoes in 1 cage were provided with only water and in the other cage, a 10% sucrose solution. After 2 days, females of both sizes and treatments were individually placed in labeled cardboard cages (9 cm diameter × 9.5 cm height) with a mesh top and were provided opportunities to imbibe human blood daily until they died. A cohort of 23 mosquitoes

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Table 1. *F*-values from the analysis of variance for the effect of size and sucrose on wing length, total number of eggs laid, and number of days survived by female *Aedes aegypti.*

Source of variation	df	*F*-values Wing length	 Number of eggs laid	 Number of days survived
Replicate	22	1.06	0.97	1.05
Size	1	161.03[1]	10.02[1]	1.66
Sucrose	1	0.07	20.05[1]	0.75
Size + sucrose	1	0.35	1.78	0.05

[1] $P \le 0.01$.

Table 2. Mean and least significant differences (LSD) values of wing length, total number of eggs laid, and days survived for small and large female *Aedes aegypti.*

Size (*n*)	Wing length (mm)	Total number of eggs	Mean survival (days)
Small (46)	2.55	432	41
Large (46)	2.95	556	36
LSD	0.06	80	7

was used for each treatment (total 46 small and 46 large). For the blood-plus-sugar group, a 10% sucrose solution was provided *ad libitum* in a cotton wick. The concentrations of sugar in floral nectar from which *Ae. aegypti* might feed in nature probably vary from 20% to 50% (Foster 1995). However, in an earlier study we determined that energy utilization by *Ae. aegypti* from *ad libitum* sugar meals is not influenced by sugar concentrations ranging from 5% to 20% (Naksathit et al. 1998). For the blood alone treatment, water was provided in a cotton wick. Wicks were replaced daily. Human blood was provided for 10 min each morning (beginning at ~10:00 to coincide with the morning peak of blood feeding detected by Strickman [personal communication] in Thailand) by human volunteers, who placed an arm on the mesh top of each cage.

Human blood feeding began when mosquitoes were separated into individual cages at ~3 days of age. In each cage, an oviposition container with 20 ml of distilled water and an oviposition substrate (brown paper towel strip measuring 3 × 8 cm) was provided throughout the experiment. Mortality and egg production were monitored daily. Eggs were counted from the oviposition substrate under a magnifying lens. Egg papers were removed each day after all mosquitoes had been given an opportunity to feed on blood. Egg papers from each treatment were allowed to embryonate for at least 7 days in an environmental chamber maintained at 25°C, 85% relative humidity, and a photoperiod of 16 h light and 8 h dark with a 20-min simulated sunset and sunrise. After embryonation, individual egg papers were submerged in distilled water and the larval hatch was monitored to verify egg fertility. Calculations for age specific survivorship (l_x), expected number of daughters (m_x), net replacement rate ($R_0 = \Sigma l_x m_x$), and intrinsic rate of growth ($r = \log_e R_0/T$) were carried out as described by Price (1984).

Results were analyzed as a complete randomized design with an analysis of variance in SAS system (SAS Institute Inc. 1989). The sources of variation are presented in Table 1. Differences among means found to be significantly different ($P \le 0.05$) were separated by a least significant differences (LSD) procedure ($P = 0.05$).

RESULTS

Table 1 shows the results of the analysis of variance that examined the effect of size and sucrose treatments on the number of eggs laid and number of days female mosquitoes survived. Mean wing lengths, average total number of eggs laid per female, and average survival days in different treatments are presented in Table 2.

Eggs laid per female: Across size categories, mosquitoes fed on blood alone laid more eggs than did those fed blood plus sugar (Tables 1 and 3). Across feeding treatments, large females laid more eggs than did small ones (Table 2). Mosquitoes fed human blood alone laid more eggs than did mosquitoes fed human blood plus sugar (Table 3 and Fig. 1).

Survival: The overall average survival time of mosquitoes in all treatments was 40 days. Mosquitoes in our laboratory study lived a relatively long time compared with expected survival for *Ae. aegypti* in their natural environment (Christophers 1960). There were no significant differences among average survival days for mosquitoes in different treatments (Tables 2 and 3 and Fig. 2).

Life table statistics: Within a size category, m_x (Fig. 1), R_0 (Fig. 3), and *r* were higher for mos-

Table 3. Mean and least significant differences (LSD) values for the total number of eggs laid and days survived for small and large female *Aedes aegypti* (*n* = 23/treatment) fed human blood alone or human blood plus sugar.

Treatment[1]	Total number of eggs	Mean survival (days)
L	671	38
L+	442	34
S	494	42
S+	370	40
LSD	113	10

[1] L, large mosquitoes fed human blood alone; L+, large mosquitoes fed human blood plus sugar; S, small mosquitoes fed human blood alone; S+, small mosquitoes fed human blood plus sugar.

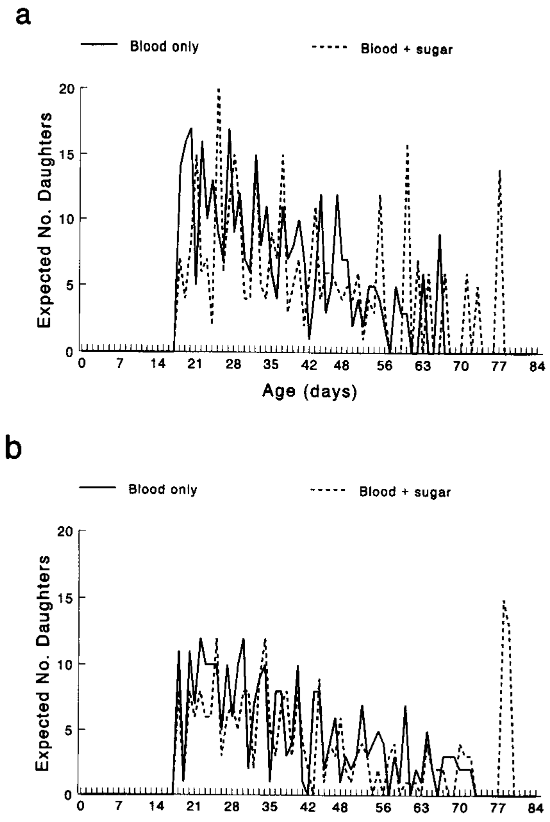

Fig. 1. Expected number of daughters for *Aedes aegypti* in different size categories (large [a] and small [b]) that were fed human blood alone versus human blood plus sucrose.

quitoes fed blood alone than for those fed blood plus sugar. Net replacement rates were 226 and 172 for small mosquitoes and 267 and 216 for large mosquitoes fed blood alone versus blood plus sugar, respectively. Intrinsic rates of growth were 0.176 and 0.160 for small mosquitoes and 0.165 and 0.152 for large mosquitoes fed blood alone versus blood plus sugar, respectively. Age-specific survivorship was not different for mosquitoes within a size class (Fig. 2).

DISCUSSION

Results from this study indicate that the fitness advantage conferred on female *Ae. aegypti* by feeding only on human blood is not modified by variation in mosquito size. Within the size classes we studied, both small and large mosquitoes fed only on human blood had higher m_x, R_0, and r and laid higher total numbers of eggs during their lifetime than did similar sized mosquitoes fed blood plus sugar (Table 1 and Figs. 1 and 3). These results agree with those reported earlier for *Ae. aegypti* that were collected as pupae from natural breeding sites in Thailand (Scott et al. 1997) and Puerto Rico (Costero et al. 1998b). Although there is an overall reproductive advantage for *Ae. aegypti* to be large (Table 2), which has been reported previously (Clements 1992), our results suggest that a reproductive advantage associated with size can be nullified by diet; i.e., when large mosquitoes feed on human blood plus sugar and small ones feed only on human blood (Table 3 and Fig. 3). Results from our life table experiments should be considered from the perspective of relative differences among mosquitoes exposed to different feeding regimes in the laboratory (Scott et al. 1997). We did not examine free-ranging mosquitoes that were exposed to natural feeding conditions (Scott et al. 1993b, Foster

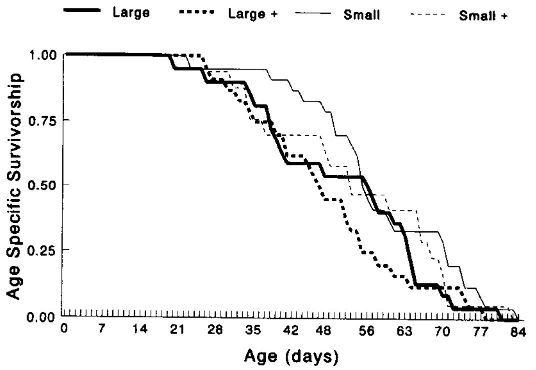

Fig. 2. Age-specific survivorship of *Aedes aegypti* in different size categories that were fed human blood alone versus human blood plus sucrose. Size and feeding status of mosquitoes are denoted as follows: large = large mosquitoes fed only human blood, large + = large mosquitoes fed human blood plus sugar, small = small mosquitoes fed only human blood, small + = small mosquitoes fed human blood plus sugar.

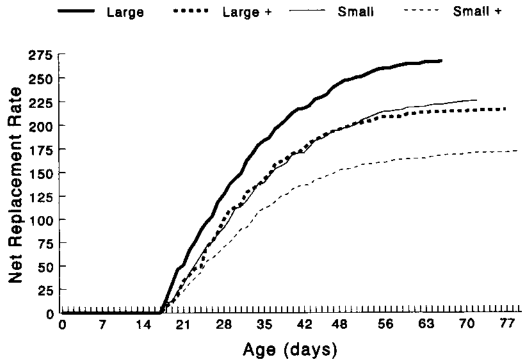

Fig. 3. Net replacement rates of *Aedes aegypti* in different size categories that were fed human blood alone versus human blood plus sucrose. Size and feeding status of mosquitoes are denoted as follows: large = large mosquitoes fed only human blood, large + = large mosquitoes fed human blood plus sugar, small = small mosquitoes fed only human blood, small + = small mosquitoes fed human blood plus sugar.

1995), energetic needs (Clements 1992), or mortality factors.

Because human blood is low in the amino acid isoleucine, mosquitoes feeding on humans may be able to use more nutrients from a blood meal for maintenance activities than if they fed on a nonhuman host (Briegel 1985). Isoleucine is essential for mosquito reproduction (Briegel 1985, 1990). Blood from nonhuman vertebrates (i.e., rodents and chickens) has comparatively higher levels of isoleucine. When *Ae. aegypti* feed on hosts with high levels of isoleucine in their blood, completion of vitellogenesis can require nutrients in the blood meal plus mobilization of maternal reserves. However, when an isoleucine-poor human blood meal is imbibed, reserves may not be needed to complete development of eggs. A portion of the nutrients in the meal can be directed to accumulation of reserves as well as vitellogenesis (Briegel 1985).

In their experiments on fecundity of *Ae. aegypti,* Day et al. (1994) used chicken blood instead of human blood to feed mosquitoes. Because chicken blood is relatively high in isoleucine, feeding on chickens may explain the reduced survival time for mosquitoes fed only blood in their study.

Nayar and Sauerman (1971, 1975a) demonstrated that *Aedes taeniorhynchus* fed blood alone survived a shorter period of time than did mosquitoes fed sugar alone. They attributed the differences in survival to a lower rate of energy reserve accumulation from blood than from sugar for egg development. However, they attributed the relatively longer survival, compared with other species, of *Ae. aegypti* in a blood alone treatment to the domestic habit of this mosquito, which requires little energy for flight compared with a normally far-ranging species like *Ae. taeniorhynchus* (Nayar and Sauerman 1973).

Although Nayar and Sauerman (1975b) reported higher fecundity for blood and sugar fed mosquitoes, they stated that there was no difference in the total number of eggs laid between blood-alone- and blood-plus-sugar-fed mosquitoes. The apparent lower number of eggs was attributed to shorter survival of blood-fed mosquitoes.

Our results indicate that if human blood is available, female *Ae. aegypti* with similar energy reserves will lay more eggs after feeding on human blood alone than when their diet includes sugar. Sugar feeding is known to decrease host-seeking behavior (Klowden, personal communication), reduce preoviposition behavior (Klowden and Dutro 1990), and delay oviposition (de Meillon et al. 1967, Hudson 1970, Shroyer and Sanders 1977, Foster and Eischen 1987, Foster 1995). The lower number of eggs laid by mosquitoes in the blood-plus-sugar treatment may also be due to the sugar meals' physical interference with blood intake. Sugar in the crop could reduce the amount of blood imbibed (Foster 1995, Klowden, personal communication) and, therefore, the number of eggs laid.

We hypothesize that the combined effect of these factors associated with survival and egg production is that female *Ae. aegypti* that feed only on human blood have a reproductive advantage over females that feed on human blood plus sugar. This observation may help explain why *Ae. aegypti* feed so often on humans (Scott et al. 1993a, 1993b; Chow-Shaffer 1997), something that makes this mosquito an efficient vector of dengue and yellow fever viruses. By feeding frequently on human blood in the peridomestic setting (Scott et al. 1993a, 1993b; Chow-Shaffer 1997), female *Ae. aegypti* obtain a selective advantage for themselves and increase fitness of the dengue and yellow fever viruses that they transmit (Scott et al. 1997, Costero et al. 1998b).

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