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A FITNESS ADVANTAGE FOR *AEDES AEGYPTI* AND THE VIRUSES IT TRANSMITS WHEN FEMALES FEED ONLY ON HUMAN BLOOD

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Abstract. Literature on arthropod-borne diseases has traditionally supported the notion that mosquito vectors maintain a feeding duality that includes vertebrate blood meals for egg development and sugar meals from plants for the synthesis of flight and survival energy reserves. *Aedes aegypti* was found to deviate from that feeding pattern by obtaining a reproductive advantage when feeding only on human blood. Female mosquitoes fed human blood alone had a greater net replacement rate and intrinsic rate of growth during all phases of their reproductive life than conspecifics fed human blood plus sucrose. Feeding frequently on human hosts during each gonotrophic cycle is necessary to avoid death due to starvation and increases exponentially the spread of *Ae. aegypti*-borne disease. Our results help explain why *Ae. aegypti* is such an unusually efficient vector of human disease; frequent biting of humans results in a high reproductive rate for vectors as well as the viruses they transmit.

Aedes aegypti (L.), the yellow fever mosquito, is the most important arthropod vector of human viral diseases. It is the principal urban vector of dengue[1] and yellow fever viruses.[2] Dengue virus infections cause more morbidity and mortality than any other arboviral disease. Each year, more than two billion people are at risk of infection. Dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) is a major cause of illness and death in Southeast Asia where 50,000–200,000 cases occur annually.[1,3] Recent *Ae. aegypti*-borne dengue epidemics in the New World, beginning in Cuba during the early 1980s, suggest an alarming increase in severe disease associated with a increasing number of infections in the Americas. The emergence of DHF/DSS during the 1950s as a major public health threat in Asia appears to be repeating itself in the Americas during the 1990s.[3]

Conventional thought regarding the feeding behavior of adult, nonautogenous, female mosquitoes, including *Ae. aegypti,* is that they require two sources of nutrition. The assumption is that all mosquitoes require a single blood meal for development of an each egg batch and periodic meals of plant sugar are necessary for synthesis of energy reserves (trehalose, glycogen, and triglycerides) used for survival and flight.[4,5] Results from recent studies on *Ae. aegypti* suggest that what is routine for most mosquito species is unusual for a species that is among the most important vectors of human disease. Female *Ae. aegypti* frequently imbibe multiple human blood meals during each egg-laying cycle[6] and are able to obtain nutrients necessary for reproduction, flight, and survival by feeding only on human blood.[5–7]

Frequent blood feeding on humans by *Ae. aegypti* is epidemiologically important because it increases host contacts and thus the opportunities for this species to acquire and transmit viral pathogens. Mathematical models of mosquito-borne diseases reveal a positive, nonlinear relationship between mosquito biting rate and the basic reproductive rate of arthropod-borne diseases; i.e., the number of new cases per infected person per day. This association suggests that even small increases in mosquito-human contact can result in relatively large increases in the number of newly infected people within a community.[8] An increase in host contact by the mosquito could, therefore, result in a selective advantage for vector-borne viruses.

It has long been recognized that female mosquitoes can synthesize glycogen and lipid from vertebrate blood. However, because sugar is a more direct and seemingly more efficient source for flight and survival energy, female mosquitoes have been assumed to maintain a blood/sugar feeding duality.[4] In fact, all mosquito species other than *Ae. aegypti* that were studied prior to our 1992 report[5] were found regularly to use sugar as food.[7]

Conversely, when *Ae. aegypti* populations are intimately associated with humans, they seldom feed on sugar, feeding instead almost exclusively on human blood. This pattern occurs even though other vertebrate host species and plants, which would provide nectar and fruit sugar, are present in the immediate environment.[5–7]

The observation that the feeding behavior of *Ae. aegypti* appears different from that of other mosquitoes, and the epidemiologic significance of this species, led us to examine factors that ultimately favor a feeding strategy in which blood is used for mosquito oogenesis, flight, and maintenance activities. We tested the hypothesis that the underlying evolutionary benefit for multiple blood feeding by this species is that it confers a reproductive advantage. That is, *Ae. aegypti* that imbibe multiple human blood meals during each gonotrophic cycle, but do not feed on sugar, are more fecund than conspecifics with a mixed diet of blood and sugar.

MATERIALS AND METHODS

We studied *Ae. aegypti* that were collected in Village 6, Chachoengsao Province in central Thailand[9] during January 1995. January is a seasonally cool and dry time of year when dengue cases do occur but are low compared with June–November when most cases are reported.[10] We limited our analyses to field-collected specimens because the conditions under which larvae develop and adults exist can profoundly affect energy reserves in adults[11] and, therefore, their ability to survive and reproduce.

Aedes aegypti–borne dengue is endemic in the study area.

Using IgM and IgG detection enzyme immunoassays, dengue virus transmission has been demonstrated among school children in Village 6 and two neighboring villages during 1990–1994 (Kittayapong P, Strickman D, unpublished data).

Throughout both experiments, mosquitoes were held under ambient environmental conditions. All mosquito cages were maintained in a cardboard box under a roof, which protected them from direct exposure to sunlight and rain.

For the life table experiment, we collected pupae on January 7–8, 1995, adults emerged on January 8–10, we placed females in individual cages on January 11, and the last mosquito died on February 24. The survival experiment began on January 20 and ended on the 30th. Mean maximum and minimum temperatures, measured in the microenvironment where mosquitoes were maintained for life table and survival experiments, were 31.7°C and 25.0°C, respectively.

Life table experiment. To test the hypothesis that mosquitoes fed only human blood have a fitness advantage over those that imbibe blood plus sugar, we carried out a life table experiment. We collected pupae from natural breeding sites within and around houses in Village 6 and held them in 50-ml, screw-top vials. We collected pupae from all detectable breeding sites; larval rearing conditions of the pupae collected were therefore assumed to be representative of the village. Adult males and females that emerged over a two-day period were housed as a group in a single, 1.8-liter, plastic cage, provided water, and allowed 1–3 days to mate randomly. After mating, females were removed, housed individually in 0.5-liter cardboard cages with a mesh top, and divided into two treatment groups of 18 mosquitoes each. One group was allowed a 10-min opportunity to imbibe blood from a human arm; blood was their only source of food. Mosquitoes were fed between 10:00 AM and noon, the peak period of *Ae. aegypti* feeding in our study area (Strickman D, Kittayapong P, unpublished data). Three different people allowed mosquitoes to feed on them, and the assignment of mosquitoes to different human hosts was randomized each day. We placed a water-soaked cotton pledget on the top of each cage between feedings. Mosquitoes in the second group received the same blood-feeding opportunity plus continuous access to a cotton pledget soaked with a 10% sucrose solution. Water and sucrose soaked pledgets were changed daily.

Cages for mosquitoes in both groups contained a plastic cup of water lined with a strip of brown paper, which served as an oviposition substrate and was labeled with the mosquito's number and date the paper was placed into the cage. At the same time each day, after mosquitoes had been offered an opportunity to imbibe blood, they were removed from cages by aspiration, paper strips were replaced, and the number of eggs laid by each female was recorded.

Therefore, each day we recorded survival (l_x) and reproduction (m_x; half the eggs laid, assuming a 1:1 sex ratio) data for each surviving mosquito until all mosquitoes had died. From this information, we calculated reproductive expectation (l_xm_x), net replacement rate (Σl_xm_x), and intrinsic rate of growth ($\log_e R_o/T$) for the two treatment groups.[12] A high net replacement rate and intrinsic rate of growth indicate a fitness advantage. Our life table statistics should be viewed in a relative sense because they do not account for mortality of immature mosquitoes before progeny become reproductively competent.

Survival experiment. To determine how long wild mosquitoes can live without food, at what point do they need to feed again, we examined survival times for mosquitoes that were deprived of food. Recently emerged adults, which were collected as pupae from natural breeding sites in Village 6 as described above, were introduced into the experiment as adults on the day of their emergence. Wild adults were captured by aspiration from natural resting sites inside houses and sorted by degree of blood engorgement.[7] Mosquitoes were grouped by category and maintained in 0.5-liter cardboard cages with mesh tops and provided water in cotton pledgets on top of their cages, but no food. An earlier study determined that ≥ 90% of all engorged mosquitoes collected at this site fed from a human host.[9]

Statistical analysis. We compared data from individual females in the life table experiment using the Kruskal-Wallace k-sample test.[13]

RESULTS

Life table experiment. Females that fed only on human blood left more offspring and reproduced more quickly than those that were offered both blood and sugar (Figure 1). Age-specific survivorship [(l_x) f = 1217.02, $P < 0.0001$], reproductive expectation [(l_xm_x) f = 14.94, $P < 0.0002$], and net replacement rate [(Σl_xm_x) f = 269.43, $P < 0.0001$] were significantly higher for the blood only group. Values for the expected number of daughters [$(m_x$ = Σeggs laid per day/females alive that day/2) f = 0.30, $P < 0.58$] were not different. The net replacement rate for mosquitoes that fed on blood alone (R_o = 77) was 45% greater than that of mosquitoes that fed on blood plus sugar (R_o = 53).

Differences in reproductive output were detected throughout the experiment. Early in the reproductive life of mosquitoes, during the first week of study, net replacement of the blood only group was almost double (17 versus 9) that of mosquitoes that fed on blood plus sugar (f = 86.68, $P <$ 0.0001). Calculations of the intrinsic rate of growth indicate that females fed only blood had a greater potential for an increase in population size (r = 0.272) than those fed mixed diets (r = 0.248).

Survival experiment. Seventy-five percent of all mosquitoes with empty guts died within 48 hr of collection when not provided food (Table 1). Fully engorged mosquitoes tended to live a day longer. After 48 hr of starvation, 90% of the engorged mosquitoes were still alive. The percentage alive did not decrease to 20% until the third day. The pattern of survival for teneral females was intermediate between those of empty and engorged females. Fifty-nine percent were alive after 48 hr and 9% were alive on the third day of starvation.

DISCUSSION

Our first effort to examine the effect of feeding *Ae. aegypti* only blood involved groups of laboratory-reared mosquitoes from a colony initiated with eggs collected in Puerto Rico.[14] The source of blood in those experiments was a live chicken. Females that were provided sugar lived longer, but

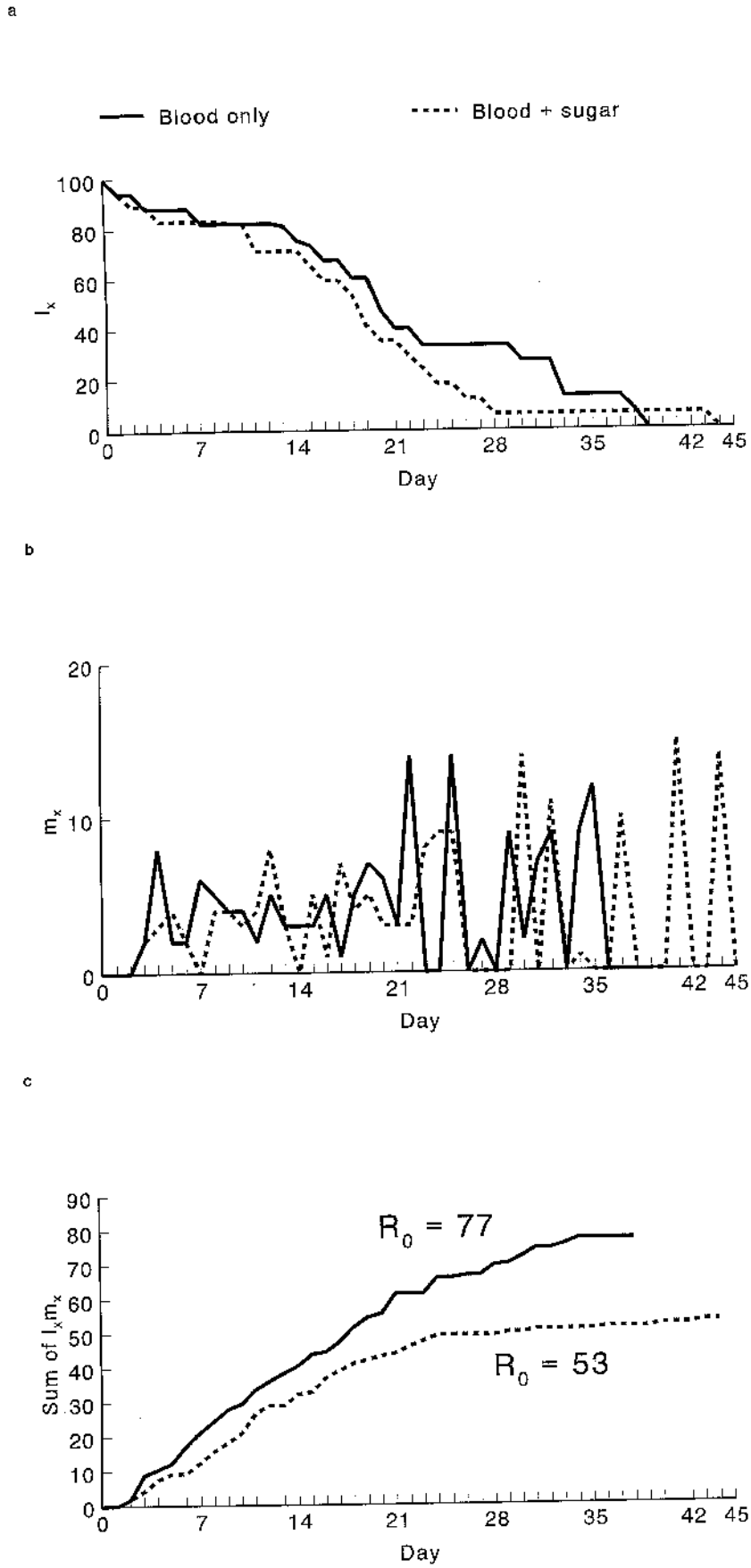


FIGURE 1. Effect of imbibing only human blood on the fitness of female *Aedes aegypti*. **a**, age-specific survivorship (l_x = percent survival). **b**, expected number of daughters (m_x = Σeggs laid per day/number of females alive that day/2, assuming a 1:1 sex ratio). **c**, net replacement rate ($\Sigma l_x m_x$).

TABLE 1

Death due to starvation of female *Aedes aegypti* that were collected from natural breeding and resting sites

Day	Number of mosquitoes found dead (% alive)		
	Recently emerged adults (n = 17)	Empty (n = 24)	Fully engorged (n = 20)
1	1 (94)	0 (100)	1 (95)
2	6 (59)	18 (25)	1 (90)
3	9 (6)	4 (8)	14 (20)
4	1 (0)	1 (4)	4 (0)
5	0	0 (4)	0
6		1(0)	
7		0	

they did not lay more eggs in their lifetime than cohorts that were fed only blood. Due to differences in blood chemistry between human and chicken hosts[4,11] and variation in the observed response when mosquitoes were housed in groups, we designed and carried out the analysis described herein in which individual mosquitoes were fed human blood.

Results from this study are consistent with the hypothesis that frequent feeding by *Ae. aegypti* on human hosts, without imbibing carbohydrates, confers a reproductive (fitness) advantage (Figure 1) and, therefore, natural selection favors that kind of feeding strategy. Frequent biting of human hosts, something that appears necessary when blood is the only source of adult food (Table 1), appears to not only benefit vector fitness but also fitness of the viruses *Ae. aegypti* transmits. In comparison with a single meal, multiple feeding in a single gonotrophic cycle increases the opportunities for vectors to contract and transmit a virus infection.[8]

Study of survival time of starved mosquitoes indicates that if wild mosquitoes do not feed every other day, they will experience an 80% probability of mortality after three days without food (Table 1). This finding is consistent with results from a histologic study of mosquitoes collected in Puerto Rico. In that investigation, 95% (38 of 40) of the *Ae. aegypti* that imbibed multiple blood meals in a single gonotrophic cycle fed at intervals $\leq$ 1 day.[6]

Absence of carbohydrate in the diet of *Ae. aegypti* may be associated with a reluctance to disperse long distances in a single flight.[15,16] Although dispersal of *Ae. aegypti* may be positively associated with a lack of oviposition sites,[17] dispersal during a brief period of time has not been documented beyond 580 m. Studies cited above indicate that most female *Ae. aegypti* do not disperse beyond 100 m. Energy reserves for flight in this species appear to be not as critical for survival as those in species that must fly long distances (i.e., a kilometer) to locate either a host or an oviposition site.

In addition to the ease with which this domestic species finds human hosts,[1,2] preference for human blood[9] may be a function of mosquito energetics.[18,19] When female *Ae. aegypti* live close to humans, they appear to synthesize most of their energy reserves from blood (Figure 1).[5,7] Frequent feeding on humans may be an efficient way to partition nutrients between maternal reserves and vitellogenesis.[19] When *Ae. aegypti* imbibe blood meals from nonhuman hosts, the caloric protein input into ovaries is greater than with human blood.[19] The amino acid isoleucine has been shown to be essential for mosquito reproduction.[11,19] Naturally low isoleucine levels in human blood are responsible for reduced conversion rates associated with human blood meals.[11,18,19] However, the high conversion rate associated with isoleucine-rich nonhuman blood allows a draw on maternal energy reserves. Feeding on humans by *Ae. aegypti* my be a way to protect maternal reserves provided to other mosquito species by carbohydrate feeding.[19] After feeding on host species with high concentrations of isoleucine in their blood, nutrients in the blood meal plus mobilization of reserves are needed to complete vitellogenesis. Isoleucine-poor human blood, however, does not result in a draw on reserves but instead allows a significant portion of the nutrients in a blood meal to be directed to, rather than taken from, reserves.[19] Nonetheless, our results indicate this does not lead to a reproductive disadvantage as might be expected.

The epidemiologically relevant fitness advantage we report for *Ae. aegypti* should be evaluated for other important domesticated mosquito vectors, especially *Anopheles gambiae,* the principal vector of malaria on the African continent. Like *Ae. aegypti,* this species is known to feed on humans, imbibe multiple blood meals during each egg-laying cycle,[20] and feed relatively infrequently on fructose.[21]

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