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Why Do Female *Aedes aegypti* (Diptera: Culicidae) Feed Preferentially and Frequently on Human Blood?

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ABSTRACT Adult female *Aedes aegypti* (L.), the vector of dengue and yellow fever viruses, have an affinity for feeding on human blood and a tendency to forego feeding on sugar. This observation challenges two tenets of mosquito biology: (1) mosquitoes imbibe plant carbohydrates for synthesis of energy reserves and blood for reproduction and (2) egg production is reduced when mosquitoes feed on human blood compared with blood from other species. Sub-optimal amounts of the amino acid isoleucine in human blood (particularly free isoleucine in plasma) are thought to be responsible for lowered egg production when human blood is ingested. We tested the hypothesis that feeding on human blood is associated with a selective advantage for *Ae. aegypti* and is an underlying reason for this mosquito's intimate and epidemiologically important relationship with human beings. Our five experiments examined the effects of different isoleucine concentrations on accumulated energy reserves, frequency of host contact, survival, and egg production. When mosquitoes imbibed blood meals over a 7- to 10-d period and were not fed sugar, increased isoleucine concentration decreased energy reserves and did not increase egg production. *Aedes aegypti* took smaller but more frequent blood meals when feeding on a low-isoleucine human host daily compared with a high-isoleucine mouse host. Previous reports that isoleucine enhances egg production were confirmed only when females were fed sugar, an unusual behavior for most domestic *Ae. aegypti* populations. Females fed human blood and water had greater age-specific survival (l_x), reproductive output (m_x), and cumulative net replacement (R_0) than cohorts fed human blood plus sugar or isoleucine-rich mouse blood with or without access to sugar. The unique isoleucine concentration of human blood is associated with *Ae. aegypti*'s unusual propensity to feed preferentially and frequently on humans—a behavior that increases this mosquito's fitness, synthesis of energy reserves, and contact with human hosts, making it an especially effective disseminator of human pathogens.

KEY WORDS *Aedes aegypti*, fitness, survival, human blood feeding, isoleucine

FOR BLOOD-SUCKING ARTHROPODS that transmit pathogens, the type of food imbibed and the frequency of feeding on human blood can influence the fitness of the pathogen as well as the arthropod vector (Scott et al. 1997), resulting in profound effects on human infection and disease. Herein, we report results consistent with the hypothesis that adult female *Aedes aegypti* (L.), the principal mosquito-vector of dengue virus worldwide and yellow fever virus in urban environments, preferentially and frequently feed on humans because a diet of human blood confers a proximate benefit in the synthesis of energy reserves and an ultimate advantage in mosquito fitness. That is, synthesis of reserves explains a physiological mechanism of how *Ae. aegypti* benefits from feeding on human blood, and increased fitness (a function of reproduction and survival) explains why natural selection favors frequent and specific contact with humans.

Aedes aegypti-borne dengue viruses cause more human morbidity and mortality than any other arthropod-borne viral disease. Each year over 2.5 billion people are at risk of infection. Recent epidemics have affected millions. Approximately 250,000–500,000 people worldwide suffer the severe consequences of infection, with a 1–5% fatality rate (Gubler 1989). Even when other hosts such as dogs, swine, rodents, bovines, and chickens are available, *Ae. aegypti* living in proximity to humans preferentially feed on human blood (Scott et al. 1993b, 2000a). This anthropophagous feeding pattern is associated with two behaviors that are unusual for females of most mosquito species; imbibing blood multiple times during each egg laying cycle and infrequent feeding on plant sugars (Scott et al. 2000b). One explanation for this epidemiologically important feeding strategy is that biochemical differences in the composition of human blood are associated with a fitness advantage compared with blood from other vertebrate species.

Geoldi (1905) first recognized differences in mosquito egg production caused by qualitative differences in host blood. He and Mathis (1934) believed that *Ae. aegypti* used human blood more efficiently than other

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types of hosts. Mathis (1934) demonstrated greater egg production per female *Ae. aegypti* offered a human host than a monkey, rabbit, or guinea pig. In these experiments, it was not clear whether females were offered sugar. Other investigators have reported opposite results; i.e., greater egg production by *Ae. aegypti* after imbibing nonhuman blood (Woke 1937a, 1937b; Lea et al. 1958; Spielman and Wong 1974; Chang 1976; Chang and Judson 1977; Nayar and Sauerman 1977; Briegel 1985) and enhanced fecundity by adding the amino acid isoleucine to human blood (Greenberg 1951; Lea et al. 1956; Chang and Judson 1979). It is important to note that in all of the latter studies, mosquitoes were fed sugar ad libitum. We now know that for wild *Ae. aegypti* in close association with humans, feeding on fructose is relatively rare (Edman et al. 1992, Van Handel et al. 1994, Martinez-Ibarra et al. 1997, Costero et al. 1998a). Therefore, we asked the following question: When sugar is eliminated from *Ae. aegypti*'s diet are there advantages associated with the chemistry of human blood that favor anthropophagy by this medically important species? Results from recent life-table studies indicate that feeding on human blood and not sugar confers a fitness advantage for *Ae. aegypti* (Scott et al. 1997, Costero et al. 1998b, Naksathit and Scott 1998, Morrison et al. 1999).

A considerable body of research indicates that the low titer of isoleucine found in human blood is the limiting factor for egg production when *Ae. aegypti* feeds on humans. Greenberg (1951) indirectly determined which components of blood are necessary for oviposition by feeding groups of *Ae. aegypti* on several types of animal blood and washed erythrocytes (rabbit, chick, sheep, and human). Feeding on washed sheep erythrocytes resulted in markedly lower egg production than whole sheep blood. To determine what component of whole sheep blood was lacking in washed erythrocytes, the author added various blood components consecutively and evaluated egg production. Significantly more eggs were produced per female after the ingestion of isoleucine supplemented sheep erythrocytes than a mixture of nine other amino acids. In another study of the nutritional factors required for egg production of *Ae. aegypti*, Lea et al. (1958) created an artificial diet containing 12 essential amino acids (arginine, cystine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), glucose, fructose, and salts. These authors also compared egg production by females offered human, cow, sheep, pig, and rabbit blood and found that females laid significantly more eggs after ingesting rabbit and pig blood than human or sheep blood (no difference was found with cow blood). Lea et al. (1958) found that when human and sheep blood was supplemented with isoleucine egg production increased to the levels of mosquitoes fed rabbit and pig blood. In these studies all females were maintained on 10% honey solution.

Dimond et al. (1956) and Bellamy and Bracken (1971) also found that certain amino acid mixtures fed per os to mosquitoes augmented egg production. Dimond et al. (1956) found eight amino acids (arginine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, and valine) that were essential for egg production. When any one of these amino acids were eliminated from the diet, no egg production resulted. Four other amino acids were found to be limiting, resulting in reduced egg production when they were removed from the diet (histidine, glutamate, cystine, and methionine). Spielman and Wong (1974) observed a positive relationship between the proportion of females initiating oogenesis and the concentration of isoleucine in serum injected via enemas into the midgut. Because isoleucine is found in low titers in human blood compared with other vertebrate species such as chickens and mice, they suggested that isoleucine might be a limiting factor in egg production. Through a series of experiments, Chang (1976) definitively demonstrated that isoleucine was the limiting amino acid in egg production. He determined that all other essential amino acids in the blood of hosts such as human and guinea pig were present in adequate amounts for egg production except isoleucine. Supplementing blood with threonine, leucine or valine, amino acids that occur in higher levels in human blood than guinea pig blood, did not reduce oviposition by mosquitoes (Chang 1976). Chang and Judson (1977) reported greater follicular resorption in mosquitoes fed human blood compared with guinea pig blood, thereby eliminating the role of isoleucine as a trigger for the endocrine release of ovarian ecdysteroidogenic hormone and demonstrating a higher rate of protein synthesis and amino acid incorporation in fat body organ cultures incubated with isoleucine.

Based on recent results regarding *Ae. aegypti*'s affinity for feeding human blood and their lack of detectable feeding on fructose (Edman et al. 1992; Chow et al. 1993; Scott et al. 1993a, 2000b; Van Handel et al. 1994, Costero et al. 1998a), we examined the energy reserve and fitness effects of a diet restricted to human blood and water and discussed the significance of that kind of feeding scenario on the transmission of pathogens such as dengue viruses. To that end, we tested the hypothesis that feeding on human blood is associated with a selective advantage for *Ae. aegypti* and is an underlying reason for this mosquito's intimate and epidemiologically important relationship with human beings. Our study had four objectives: (1) To reexamine, in light of the new findings mentioned above on *Ae. aegypti* feeding behavior, results from previous studies indicating that *Ae. aegypti* fed isoleucine poor human blood produce fewer eggs than those fed equivalent volumes of isoleucine rich blood. (2) To determine the effects of isoleucine poor human blood on the synthesis of energy reserves by *Ae. aegypti.* (3) To determine how sugar feeding effects egg production. (4) To determine the effects of blood meals from different host species with high versus low concentrations of isoleucine on *Ae. aegypti* fitness. We defined fitness as the relative ability of mosquitoes to produce offspring throughout their lifetime (Price 1997, Futuyma 1998) and measured the net replacement rate (R_o) and intrinsic rate of increase (r), which account for the interaction between reproduction and survival.

Materials and Methods

For all experiments, larvae from a Thai strain of *Ae. aegypti* originally collected from Chachoengsao Province (13° 38′ N, 101° 18′ E; with < 50 laboratory generations from the wild type) were reared at 27 ± 1°C and a photoperiod of 14:10 (L:D) h to obtain adults of uniform body size (Day et al. 1994). After eclosion, mosquitoes were housed in 35-cm^3 rearing cages, at 75% RH and the same temperature and photoperiod as the immatures. Adults were given access to water but not sugar before experimentation.

Experiment 1: High and Low Isoleucine Human Blood Fed Through Artificial Membranes. In our first experiment, we determined the effect of experimentally varying the concentration of isoleucine in human blood on the synthesis of energy reserves and the production of eggs in a single gonotrophic cycle. This experiment determined, if without feeding sugar to mosquitoes, we could reproduce results from previous studies, which indicated that mosquito egg production was correlated positively with the concentration of isoleucine in vertebrate blood.

Mosquitoes. At 3 d of age adult females were transferred to Plexiglas cages (18 by 18 by 30 cm).

Preparation of Blood and Artificial Feeding. Using the method of Briegel (1985), 1.3 mg of L-isoleucine (Sigma, St. Louis, MO) were dissolved in 2 ml of a 9% sodium chloride solution and added to 48 ml of human plasma. Two 1.5-ml aliquots of plasma (one isoleucine-supplemented and one unsupplemented) were removed and frozen for later baseline isoleucine quantitation. Median baseline isoleucine ranges for human plasma were obtained from the New England Medical Center Pediatric Amino Acid Laboratory (Boston, MA). Fresh frozen plasma and expired red blood cells were purchased from the American Red Cross. Blood cells and plasma were combined in a 1:1 ratio. Reconstituted blood was offered artificially through a membrane (Kwik-Fill hog intestine, F.B. Casing, Brooklyn, NY). Blood was warmed to body temperature by circulating water from a 37°C bath through a glass-jacketed feeder for 30 min. The feeding membrane was placed against the mesh top of mosquito cages, allowing females to feed through the mesh. Mosquitoes were offered a blood meal once each day (20 min) for 7 d. Mosquitoes that did not feed on the first day were eliminated. Mosquitoes to be analyzed for baseline energy levels were removed just before blood feeding on the first day and frozen at −80°C.

Blood Meal Weights. Ten blood-fed and 10 unfed mosquitoes were cold-anesthetized and weighed individually with an electrobalance. Weights of ingested blood were estimated by subtracting the mean weight of empty mosquitoes from the weight of engorged specimens. Because mosquitoes were chilled immediately after feeding and weighed within 30 min, we assume that losses due to diuresis were negligible (Clements 1992).

Egg Production. No oviposition medium was provided in this experiment, females were forced to retain their eggs over the entire blood-feeding period. After the seventh day of feeding, ovaries from each female were dissected in phosphate buffered saline and developing stages III–IV and mature stage V ovarioles (Christophers 1960) were counted.

Mosquito Preparation and Nutritional Analysis. Cadavers and ovaries from dissected mosquitoes were placed in separate test tubes. Liquid from dissections was placed in the tube with the body. Samples were dried in an oven at 70°C for 2 h. Methods of Van Handel and Day (1988) were used to determine the amount of glycogen, sugar and lipid in each mosquito's body and pair of ovaries.

Plasma Isoleucine Quantitation. Unsupplemented and isoleucine-supplemented plasma shipped to the Protein Chemistry Laboratory at the University of Texas were thawed, and 200 μl of plasma and 800 μl of 3.75% sulfosalicyclic acid with 2.5 nmoles of 4-pyridylethyl-L-cysteine (4-PEC) were mixed and vortexed. Samples were refrigerated for 30 min and then centrifuged at 4,120 × *g* for 30 min. Supernatant was filtered through a 20-μm filter (13 mm, Acrodisc PTFE) and 100 μl was loaded into sample loops of a Beckman 6300 amino acid analyzer (Beckman, Fullerton, CA); 4-PEC was used as the internal standard.

Experiment 2: High and Low Isoleucine in Artificial Blood Meals Versus Intact Vertebrate Hosts. Using the same design as our first experiment, we next investigated how different concentrations of isoleucine in artificial blood meals versus intact vertebrate hosts affect reserve synthesis and egg production. We determined the extent to which results from our first experiment with artificially manipulated human blood can be extended to different vertebrate species whose blood naturally varies in isoleucine concentration.

Mosquitoes. At 3 d of age females were transferred to cylindrical cardboard containers (0.5-liter capacity) with mesh tops.

Blood Preparation. Artificial meals were prepared and offered through a membrane as described for experiment 1. For natural blood meals, mosquitoes in cardboard containers were allowed to feed on a human forearm (L.C.H.) or on a chick secured, abdomen-side-down, over the mesh end of the container. Blood meal weights, developing and mature oocytes (females were not provided with an oviposition medium), nutritional reserves, and plasma isoleucine were measured as described in experiment 1. Chick and human plasma from live hosts in this experiment were not submitted for amino acid quantitation.

Experiment 3: Comparison of Blood from Intact Human and Mouse Hosts. This experiment determined the effect of isoleucine concentration on reserve synthesis and egg production when only intact hosts were used as a source of blood. In this experiment, mosquitoes where allowed to lay their eggs rather than forced to retain them. In addition to providing an oviposition medium, the amounts of reserves were examined at three different times, rather than just once, during the 10-d study period.

Mosquitoes. Three-day-old adult females were placed individually into containers (237 ml). Small cups (59 ml) lined with paper toweling and filled with

30 ml of distilled water were placed inside each container as an oviposition site. Water moistened cotton pledgets were provided during the first 3 d after ecdysis.

Wing Length Measurements. Mosquito wing lengths provide an estimate of body size (Christophers 1960). The right wing from each female was removed and mounted on a glass microscope slide with clear adhesive tape. Each wing was measured following the methods of Nasci (1990).

Blood Feeding. Mosquitoes were allowed to feed on either a human forearm (L.C.H., low isoleucine) or on a restrained laboratory mouse (high isoleucine). Mosquitoes that did not feed on blood during the first day were eliminated. Feeding was confirmed by shining a light on one side of the lateral aspect of each mosquito and viewing the illuminated abdomen through the pleural membrane from the opposite side. Fresh blood meals (large and small) were evident as bright red in the distended abdomen. Deposited eggs were counted each day after blood feeding and a fresh paper towel was placed in the oviposition cup. Mosquitoes were offered blood at the same time each day for 15 min for 10 consecutive days. Groups of mosquitoes (from each host) were removed on days 2, 6, and 10 of the experiment and frozen at −80°C. Blood meal weights and daily egg production were measured as described for experiment 1.

Plasma Isoleucine Quantitation. Human blood was drawn by the University of Massachusetts Health Center as described above and mouse blood was drawn by personnel at the University of Massachusetts Animal Care Facility. Plasma and cells were separated by centrifugation for 20 min at 2,019 × *g* and plasma was placed in microcentrifuge tubes and frozen at −70°C. Frozen plasma was shipped to the Protein Chemistry Laboratory at the University of Texas where it was thawed and analyzed for isoleucine as described for experiment 1.

Energy Reserves in Eggs. Eggs deposited by individual females were analyzed for glycogen, sugar, and lipids following the methods described for mosquito bodies and ovaries in experiment 1. Total nutrient values were divided by the number of eggs in each sample to obtain glycogen, sugar, and lipid values per egg. These data were compared for eggs produced by females that fed on human versus mouse blood.

Experiment 4: Effect of Sugar Feeding on Egg Production. In experiments 1–3 we did not detect differences in egg production for mosquitoes offered various forms of low or high isoleucine blood and not fed sugar. Those results were contrary to reports from earlier studies that indicated that after access to sugar a single human blood meal was inferior for mosquito egg production compared with blood from other vertebrate species. We, therefore, carried out a fourth experiment to determine if by adding sugar to the diet we could restore the sub-optimal qualities of human blood for egg production.

Mosquitoes. Teneral females were transferred individually to cardboard containers (237 ml) fitted with mesh lids. Experimental mosquitoes were divided into two cohorts: one was provided water and the other a 20% sucrose solution in saturated cotton pledgets on top of the cages. Oviposition cups lined with paper towels were added to containers as described for experiment 3.

Blood Feeding. Mosquitoes were allowed to feed on a human forearm (L.C.H.) or a restrained mouse as described for experiment 3. Mosquitoes were offered a single blood meal when they were 3 d old. Females that did not feed on blood were discarded. Blood meal weights were measured as described for experiment 1.

Egg Production. The number of eggs laid was recorded daily as in experiment 3 for each female for 5 d after they imbibed the blood meal.

Experiment 5: Life-Table Analysis of Fitness Effects. Our last experiment was a life-table experiment, which determined how the concentration of isoleucine in host blood effects *Ae. aegypti* fitness. We tested the prediction that a high isoleucine blood meal without access to sugar could result in death by starvation, because high isoleucine concentrations lead to the use of nutrients in a blood meal as well as available maternal reserves for egg development. Conversely, nutrients in a low isoleucine blood meal (without access to sugar) can be partitioned between egg development and synthesis of maternal reserves that are necessary for nonreproductive maintenance activities (Briegel 1985).

Mosquitoes. Individual females were placed into cardboard containers (237 ml) fitted with mesh lids. From saturated cotton pledgets, one cohort was offered a 20% sucrose solution and the other only water. Oviposition sites were provided as described for experiment 3.

Blood Feeding. Mosquitoes were given an opportunity to imbibe blood from a human (L.C.H., low isoleucine) or mouse (high isoleucine) daily for 15 min until all mosquitoes had died. Blood meal weights were measured as described for experiment 2, and egg production was recorded daily for each mosquito, as in experiment 3.

Plasma Isoleucine Quantitation. Human and mouse blood were collected as described for experiment 3 and shipped to the Protein Structure Laboratory at the University of California, Davis, where they were thawed and each sample was analyzed for isoleucine as described for experiment 1.

Data Analysis. Data for each experiment were checked for conformation to the assumptions of normality and homoskedasticity. When the assumption of equality of variances was violated with normal data, a *t*-test using unpooled variances was employed. Other comparisons of non-normal data were conducted with the nonparametric Mann–Whitney *U* test and Kruskal–Wallis one-way analysis of variance (ANOVA) (Sokal and Rohlf 1981). To estimate the relative fitness of mosquitoes fed different types of host blood, the age specific survival (l_x), expected number of daughters (m_x), net replacement rate (R_0), and intrinsic rate of increase (r) were calculated (Southwood 1978). We first estimated r from the natural log of the net replacement rate divided by the

Table 1. Total eggs (developing Christophers' stage III–IV oocytes plus eggs deposited) per female *Ae. aegypti* offered low or high isoleucine (ILE) blood without sugar

Experiment/host	Low isoleucine blood		High isoleucine blood	
	No. ♀♀	No. eggs (mean ± SE)	No. ♀♀	No. eggs (mean ± SE)
Exp. 1 artificial (human)	18	64.4 ± 4.3a[a]	28	62.8 ± 3.6a[b]
Exp. 2 artificial (human)	9	55.8 ± 10.6b	15	52.0 ± 4.4b
Exp. 2 natural (low ILE human or high ILE chick)	34	84.1 ± 5.3c	25	83.6 ± 5.4c

Means followed by the same letter were not significantly different from each other. a, Experiment 1: *t*-test, $t = 2.02$, df = 44, $P = 0.78$, b, c, Experiment 2: ANOVA, $F = 8.59$; df = 3, 78; $P < 0.01$.

[a] Plasma ILE concentration = 57.0 nm/ml.

[b] Plasma ILE concentration = 172.2 nm/ml.

generation time T_c. The actual value of r was calculated in an Excel spreadsheet using successive approximation. Life-table parameters were compared among cohorts with the Kruskal–Wallis one-way ANOVA.

Results

Experiment 1: High and Low Isoleucine Human Blood Fed Through Artificial Membranes. Baseline isoleucine concentration in normal human plasma (low isoleucine) was 57.0 nm/ml, which was near the New England Medical Center Pediatric Amino Acid Laboratory's median baseline value of 66.3 nm/ml plasma. Plasma in supplemented (high isoleucine) blood meals contained approximately three times this amount (172.2 nm/ml).

We did not detect significant differences ($P > 0.05$) in the number of eggs produced per mosquito for the cohorts fed low or high isoleucine blood meals (Table 1). However, significantly more glycogen (mean ± SE; low = 30.9 μg ± 4.0; high = 17.6 μg ± 1.0; $t = 3.22$, df = 19, $P = 0.002$) and sugar (low = 6.3 μg ± 2.4; high = 2.4 μg ± 0.3; $t = 2.03$, df = 44, $P = 0.02$) were detected in the total body (cadaver and oocytes) of mosquitoes fed the low isoleucine blood compared with the high isoleucine diet (Fig. 1). A significant difference was not detected in lipid concentrations between the two cohorts (Fig. 1).

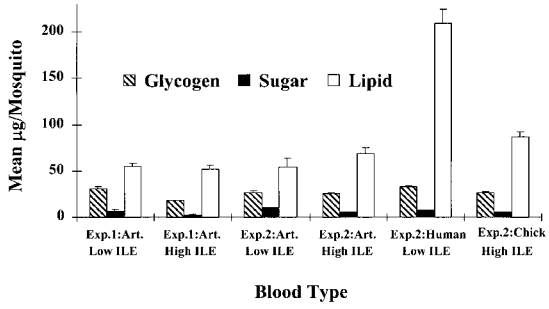

Fig. 1. Mean glycogen, sugar, and lipid content (μg) per female *Ae. aegypti* fed blood with a high or low isoleucine (ILE) concentration artificially (Art.) through a membrane or from natural hosts in two different experiments (experiments [Exp.] 1 and 2). Bars represent the standard error of the mean.

Experiment 2: High and Low Isoleucine in Artificial Blood Meals Versus Intact Vertebrate Hosts. Again, no significant difference was found in egg production between females artificially fed low isoleucine human blood (55.8± 10.6 eggs) compared with isoleucine supplemented human blood (52.0± 4.4 eggs) (Table 1). The same was true for egg production between females fed on a natural human host (low isoleucine = 84.1± 5.3 eggs) versus a chick (high isoleucine = 83.6± 5.4 eggs) (Table 1). Females fed on natural hosts developed significantly more eggs (Table 1) and took noticeably larger blood meals than artificially fed females (mean blood meal weight, low isoleucine natural human = 2.50 mg ± 0.39; high isoleucine natural rodent = 2.59 ± 0.31; artificial low isoleucine = 1.90 ± 0.35; and artificial high isoleucine = 1.71 ± 0.25).

No differences in glycogen reserves were detected between mosquitoes fed high and low isoleucine in artificial blood meals (Fig 1). Marginally significant differences in mean glycogen concentrations were found between mosquitoes fed human blood (32.7 μg ± 2.7) compared with chicken blood (high isoleucine) (26.4 μg ± 3.0) ($t = 1.54$, df = 56, $P = 0.06$). Differences were slightly more pronounced when bodies alone (without the oocytes) were compared (high = 20.9 μg ± 2.2; low = 15.8 μg ± 2.0; $t = 1.65$, df = 56, $P = 0.05$).

Mosquitoes fed low isoleucine artificial blood had significantly greater mean sugar reserves (10.7 μg ± 2.6) than those fed high isoleucine (5.7 μg ± 0.7; $t = 1.82$, df = 9, $P = 0.05$); a result attributable to body sugar levels rather than to ovaries. The human blood fed cohort had greater sugar reserves (6.9 μg ± 1.1) than mosquitoes fed chick blood (4.8 μg ± 0.7), although the difference was slight ($t = 1.62$, df = 51, $P = 0.056$) (Fig. 1).

Lipid reserves in mosquitoes fed natural human blood (210.0 μg ± 14.2) were almost three times greater than in the cohort fed natural chick blood (87.3 μg ± 4.7, $t = 8.18$, df = 39, $P < 0.00001$). No lipid reserve differences were found among mosquitoes fed artificial blood meals (Fig. 1).

Experiment 3: Comparison of Blood from Intact Human and Mouse Hosts. Mean isoleucine content in human plasma was 42.3 nm/ml (range, 41.8–42.8) compared with 117.9 nm/ml (range, 96.7–139.0) in

Table 2. Free plasma amino acid concentrations in blood samples from experiment 3

Essential amino acid[a]	Human plasma[b] nmol/ml (female)	Human plasma[c] nmol/ml (female)	Mouse plasma nmol/ml (male)	Mouse plasma nmol/ml (female)
Isoleucine	41.8	42.8	139.0	96.7
Arginine	104.1	97.8	441.4	179.4
Leucine	87.4	87.7	245.4	189.5
Lysine	184.0	186.2	299.2	316.2
Phenylalanine	67.1	61.5	100.5	84.6
Threonine	123.1	122.1	183.0	182.4
Valine	174.5	191.3	312.9	227.8

[a] Tryptophan is destroyed by analysis and, therefore, could not be quantitated.
[b] Sample 1 (experiment 3).
[c] Sample 2 (experiment 3).

mouse plasma. The variability in isoleucine concentrations in the mouse plasma represented differences according to sex. Male mice were at the high end of the range (3 times human plasma), whereas females were at the low end (2 times human plasma) (Table 2). Equal numbers of male and female mice were used alternately as blood sources for mosquitoes.

The mean wing length of mosquitoes was 2.85 mm ± 0.02 ($n = 40$). Average daily mortality in the human-fed cohort (8%) was significantly lower than in the mouse-fed cohort (14%; $t = -1.74$, df = 18, $P = 0.049$).

Aedes aegypti in the mouse-fed cohort took significantly larger blood meals (mean weight = 1.64 mg ± 0.13) over the 10 d feeding period than did those in the human-fed cohort (1.20 mg ± 0.11; $t = -2.54$, df = 16, $P = 0.01$). Overall, significantly higher daily rates of engorgement were observed among mosquitoes fed human blood (52.4%) compared with those fed mouse blood (39.7%). Initially, daily engorgement rates (percentage of total feeding) on the two hosts were similar. Significantly more of the mosquitoes exposed to a human than rodent host fed on days 5–10 (9–14 d of age) ($\chi^2 = 6.45$, df = 1, $P = 0.011$). In the human-fed group, 69% fed on days 1–4 and 45% fed on days 5–10, compared with 63% feeding on days 1–4 and 23% on days 5–10 in the rodent-fed group (Table 3).

The total number of eggs (retained plus deposited) produced by mosquitoes that imbibed human and mouse blood were not significantly different (range, 24–29 eggs per mg of blood ingested; Table 4).

Human-fed mosquitoes killed on days two and six had significantly more glycogen in their bodies (day 2 = 25.5 μg ± 3.08, $t = 2.23$, df = 37, $P = 0.02$; day 6 = 22.9 μg ± 0.28, $t = 1.96$, df = 33, $P = 0.03$) than mouse-fed females at two (mean = 16.7 μg ± 2.5) and 6 d (mean = 13.7 μg ± 0.4). On day 10, the mouse-fed cohort (11.3 μg ± 1.8) had significantly more glycogen reserves than the human-fed cohort (6.5 μg, $t = -2.36$, df = 26, $P = 0.013$), but this difference was primarily due to a greater amount in the ovaries (mouse = 2.9 μg ± 1.0, human = 1.5 μg ± 0.5; Fig. 2A).

Significantly more sugar was detected in the bodies of human-fed than mouse-fed mosquitoes on day 2 (human = 5.31 μg ± 1.3; mouse = 2.7 μg ± 0.3; $t = 1.94$, df = 20, $P = 0.03$) and day 10 (human = 2.9 μg ± 0.4; mouse = 2.2 μg ± 0.2; $t = 1.80$, df = 24, $P = 0.04$), but not on day 6 (human = 6.9 μg ± 1.4; mouse = 4.6 μg ± 0.9; $t = 1.33$, df = 31, $P = 0.09$) (Fig. 2B).

Greater lipid reserves were detected in the bodies of human-fed mosquitoes on day 2 (human = 95 μg ± 10.0; mouse = 65.2 μg ± 13.1; $t = 3.02$, df = 38, $P = 0.002$) and day 6 (human = 112.3 μg ± 10.6; mouse = 86.8 μg ± 12.6; $t = 2.17$, df = 32, $P = 0.019$) (Fig. 2C), but not on day 10. Even more dramatic were the nutritional differences when mosquitoes removed on days 2, 6, or 10 were combined (human $n = 52$, mouse $n = 45$). Significantly greater glycogen ($t = 2.79$, df = 87, $P = 0.003$), sugar ($t = 1.85$, df = 82, $P = 0.03$), and lipid reserves ($t = 2.66$, df = 95, $P = 0.005$) were detected per milligram of blood ingested in females that fed on a human (Fig 3).

No significant differences were found in mean glycogen (human = 0.49, rodent = 0.43; $t = 2.00$, df = 61, $P = 0.20$), sugar (human = 0.06, rodent = 0.03; $t = 1.99$,

Table 3. Percent of *Ae. aegypti* females feeding daily on a human versus rodent host (*n*)

Day	Exeriment 3		Experiment 5			
	Human	Rodent	Human	Rodent	Human and sugar	Rodent and sugar
1	100 (110)	100 (110)	100 (72)	100 (70)	100 (74)	100 (73)
2	79 (107)	77 (78)	31 (62)	29 (68)	19 (74)	39 (72)
3	46 (60)	40 (44)	46 (59)	8 (64)	15 (73)	9 (70)
4	41 (55)	42 (42)	44 (59)	8 (60)	13 (72)	12 (67)
5	81 (48)	55 (39)	53 (59)	54 (52)	34 (71)	16 (63)
6	56 (35)	34 (17)	36 (59)	23 (43)	23 (66)	50 (61)
7	34 (24)	31 (16)	30 (56)	28 (40)	5 (62)	11 (57)
8	57 (22)	36 (14)	42 (52)	19 (31)	7 (60)	10 (49)
9	32 (17)	25 (12)	44 (50)	38 (24)	22 (58)	11 (35)
10	47 (17)	17 (12)	26 (46)	11 (18)	26 (46)	14 (28)

Table 4. Total eggs (developing Christophers' stage III–IV oocytes plus eggs deposited) per female *Ae. aegypti* per mg of human (low isoleucine) or mouse (high isoleucine) blood ingested

Day removed	No. ♀♀	Human host eggs/mg blood (mean ± SE)	No. ♀♀	Mouse host eggs/mg blood (mean ± SE)
2	14	26.7 ± 1.4	14	24.0 ± 1.9
6	20	27.7 ± 2.3	13	29.1 ± 3.9
10	16	26.0 ± 2.8	12	27.9 ± 5.7
Total	50	26.9 ± 1.3	39	26.0 ± 2.5

Females were removed after 2, 6, and 10 d of blood feeding. Sugar was not offered and differences were not significant.

df = 61, $P = 0.12$), and lipid reserves (human = 0.29, rodent = 0.21; $t = 2.01$, df = 46, $P = 0.30$) per egg produced by females offered human or rodent blood (Fig. 4).

Experiment 4: Effect of sugar feeding on egg production. We refer to those mosquitoes offered blood with access to sugar as "blood plus sugar" and those offered blood with no access to sugar as "blood alone." Mosquitoes fed human blood alone ingested significantly more blood (3.92 mg ± 0.18) than those fed human blood plus sugar (2.38 mg ± 0.11) or mouse blood plus sugar (2.52 ± 0.09, $P < 0.0001$, Kruskal–Wallis test). Contrary to our previous results demonstrating that over several days human-fed females took smaller and more frequent meals (Table 3), no difference in meal size was found when mosquitoes fed on different hosts ingested only one blood meal (Fig. 5).

Egg production per female was significantly greater for mosquitoes fed mouse blood plus sugar (Table 5) compared with the other three cohorts (ANOVA, $F = 66.9$; df = 3, 132; $P < 0.0001$). Egg production per mg of ingested blood for the mouse blood alone and human blood alone cohorts was not significantly different. Greatest egg production was found with those females offered mouse blood plus sugar followed by those offered human blood plus sugar (Table 5).

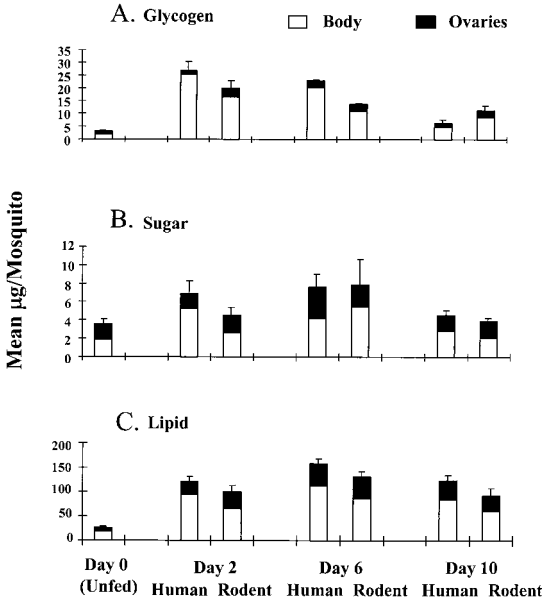

Fig. 2. Mean glycogen (A), sugar (B), and lipid (C) content (µg) per *Ae. aegypti* that imbibed human or mouse blood from an intact host without access to sugar (experiment 3). Bars represent the standard error of the mean.

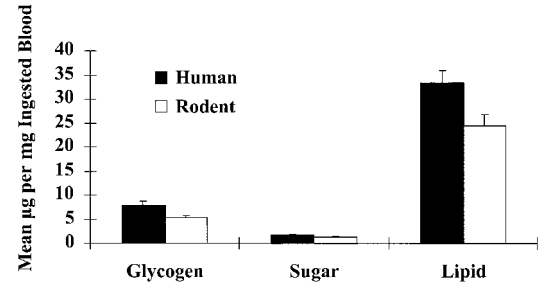

Fig. 3. Mean glycogen, sugar, and lipid content (µg) per milligram of ingested blood in female *Ae. aegypti* fed naturally on intact human or mouse hosts without access to sugar (experiment 3). Bars represent the standard error of the mean.

Experiment 5: Life-Table Analysis of Fitness Effects. The mean isoleucine content in the human plasma was 74.8 nm/ml (1 sample) compared with a mean of 130.4 nm/ml (range, 118.1–130.4) in mouse plasma. During the first 10 d, daily frequency of blood feeding was greater among mosquitoes fed human blood alone (44%) compared with those fed mouse blood alone (31%) ($P = 0.03$, Mann–Whitney test; Table 3).

A 1:1 ratio of male to female offspring was assumed for life-table calculations. We fed 63, 71, 68, and 70 females on human blood alone, human blood plus sugar, mouse blood alone, or mouse blood plus sugar, respectively. *Aedes aegypti* fed human blood alone had the highest age-specific survival (l_x), followed by those fed human blood plus sugar, mouse blood plus sugar, and mouse blood alone (Fig. 6A). The difference between mosquitoes fed human blood alone and mouse blood alone was significant ($P < 0.003$, Kruskal–

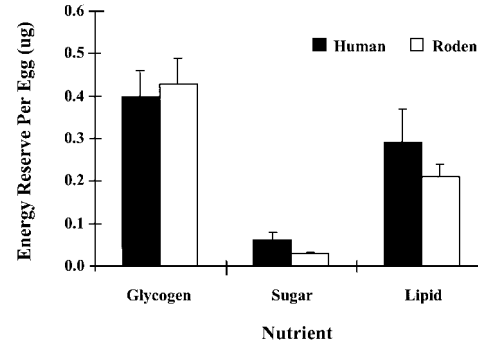

Fig. 4. Mean glycogen, sugar, and lipid content (µg) in each egg produced per mg of human or mouse blood ingested by female *Ae. aegypti* (experiment 3). Bars represent the standard error of the mean.

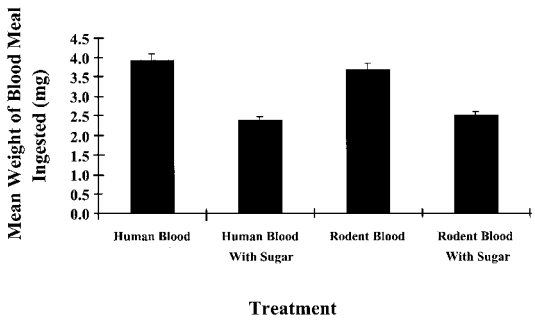

Fig. 5. Blood meal volumes ingested by female *Ae. aegypti* offered single human or mouse blood meal with and without sugar (experiment 4). Bars represent the standard error of the mean.

Wallis test). Estimated reproductive output per day (m_x; $P < 0.01$, Kruskal–Wallis test) and net replacement rate (R_0; $P < 0.0001$, Kruskal–Wallis test) also was greater for females fed human blood alone than for the mouse-fed mosquitoes (Fig. 6 B and C). Although mosquitoes fed mouse blood plus sugar produced significantly more eggs, their net replacement rate was lower than for the two groups fed human blood. Intrinsic rate of increase also was lower for mosquitoes fed mouse blood plus sugar ($r = 0.487$) than for the other three cohorts (human blood alone, $r = 0.734$; human blood plus sugar, $r = 0.730$; mouse blood alone, $r = 0.713$). Mosquitoes fed mouse blood alone had the shortest life-span of the four cohorts (Fig. 6A).

Discussion

Aedes aegypti benefit from an accumulation of more energy reserves and a fitness advantage when they ingest low (human) rather than high (rodent or chicken) isoleucine blood. This difference in energy reserves was less pronounced when females artificially were offered unsupplemented and isoleucine-supplemented human blood, indicating that there may be other components in human blood in addition to low isoleucine levels that contribute to the accumulation of energy reserves. The most dramatic differences in energy reserves were the greater accumulation of triglycerides (lipids) in females fed human blood compared with chicken or mouse blood. We predict that the accumulation of triglyceride reserves is more important for a species like *Ae. aegypti* because of its sedentary lifestyle. Triglycerides are the main source of energy for resting mosquitoes, whereas carbohydrates from plants or honeydew are a more efficient substrate for the synthesis of sugar and glycogen reserves used for flight (Nayar and Van Handel 1971). *Aedes aegypti* can synthesize triglycerides from blood (Van Handel 1965), does not typically fly long distances, and may remain for long periods in the same house where hosts, mates, and oviposition sites are readily available within meters of each other (Morland and Hayes 1958, McDonald 1977, Trpis and Hausermann 1986, Edman et al. 1998). The sugars detected by our assays were not from an exogenous source and were most likely trehalose (Van Handel 1985, Clements 1992).

Table 5. Effect of sugar feeding on egg production after a single high or low isoleucine host blood meal (experiment 4)

Cohort	No. ♀♀	Mean no. eggs per mg ingested blood ± SEM
Human blood alone	36	13.6 ± 1.5b
Human blood and sugar	36	32.4 ± 1.9c
Mouse blood alone	27	19.8 ± 1.8b
Mouse blood and sugar	37	47.7 ± 2.2d

Means followed by the same letter are not significantly different from each other (ANOVA, $P < 0.0001$; df = 3, 132; $F = 66.9$).

Increased synthesis of energy reserves by mosquitoes fed human blood compared with mouse blood and higher mortality among those offered mouse blood and not fed sugar, support the hypothesis that natural selection favors female *Ae. aegypti* that feed on human blood without feeding on sugar (Scott et al. 1997, Costero et al. 1999). A feeding strategy restricted to human blood requires diversion of at least part of the nutrients in the meal to synthesis of energy reserves (Briegel 1985), which would contribute to increased female longevity and lifetime production of offspring as indicated by our life-table experiment.

Of all the host species (human, dog, cat, rodent, bovine, porcine, and avian) available to *Ae. aegypti* in its natural rural and urban habitats, humans are often the most abundant and consistently available. Forage indices from a Thai study determined that *Ae. aegypti* fed on humans more than any other hosts available, including dogs, bovines, cats, chickens, and swine (Scott et al. 1993b). Selective and frequent feeding on humans, coupled with infrequent feeding on sugar, appears to be an adaptation to a highly domesticated lifestyle that only a few mosquito species have adopted. Domesticity in *Ae. aegypti* may have evolved in North Africa at a time when the environment became increasingly arid (Petersen 1977). This change in the environment may have produced an isolated population of *Ae. aegypti* that was forced to adapt to the dry conditions by breeding in human-made water storage containers. Preference for blood from a single species may have been an adaptation for living in arid or indoor situations where sugar is scarce and human blood is plentiful. Peridomestic mosquito species like *Ae. aegypti* and *Anopheles gambiae* (Giles) sensu lato, which feed preferentially on humans and forego feeding on plant carbohydrates, appear to have adopted this strategy (Edman et al. 1992, Beier 1996, Scott et al. 1997). Interestingly, these two species are among the most important vectors of human pathogens worldwide. Another mosquito with an anthropophagous feeding pattern that should be investigated, with respect to the affect of host blood type on nutrition and survival, is *Culex pipiens quinquefasciatus* (Say), an important vector of filariasis and several arboviruses

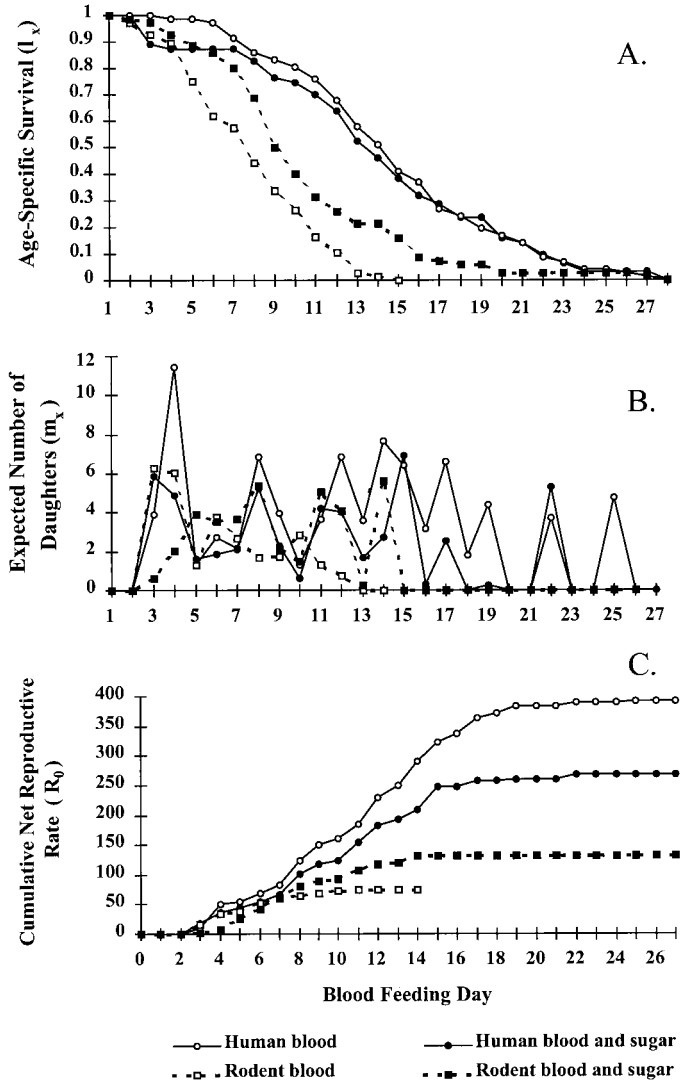

Fig. 6. Life table statistics for *Ae. aegypti* allowed to feed on a human or mouse host daily with or without access to sugar. (A) Age specific survival (l_x). (B) Expected number of daughters (m_x). (C) Cumulative net reproductive rate ($R_0 = \Sigma\, l_x m_x$) (experiment 5).

(Marshall 1988, Sucharit et al. 1988, Tsai and Mitchell 1988, Hati et al. 1989).

Results from our fourth experiment explained the lack of agreement between our first three experiments and reports from the literature that *Ae. aegypti* imbibing mouse blood produce significantly more eggs than females fed an equivalent amount of human blood (Woke 1937a, 1937b; Lea et al. 1958; Chang 1976; Chang and Judson 1977, 1979). In the first three experiments we did not feed sugar to mosquitoes and we could not confirm our predecessors results. We detected no difference in egg production for mosquitoes (1) fed artificial human blood supplemented and unsupplemented with isoleucine or (2) when we fed them on intact hosts whose blood varied naturally in isoleucine concentration. After our first experiment we were concerned that smaller meals taken by mosquitoes fed artificial versus natural blood meals might have influenced our results. Therefore, we carried out follow-up experiment 2, in which blood containing different concentrations of isoleucine was offered from an intact, natural host (human or chick) as well as artificially through a membrane. Again, regardless of how the blood was prepared and presented, no differences in egg production were detected. In experiment 3, measurements of mean blood meal weights were used to calculate the number of eggs produced per unit of blood ingested. We also tracked egg production over a broader period than in our previous two experiments and investigated the cumulative effect of blood feeding during more than a single gonotrophic cycle. Again, no differences in egg pro-

duction were detected based on the concentration of isoleucine in the blood meal. Results from our first three experiments, therefore, conflicted with the widely held belief that egg production is correlated positively with the isoleucine concentration in host blood (Chang and Judson 1977, Briegel 1985, Clements 1992).

It is common practice to provide sugar to experimental mosquitoes during laboratory studies (Gerberg 1970). However, in the past 10 yr it has become apparent that when *Ae. aegypti* live in the dwellings of their human hosts, it is relatively rare to capture females with detectable amounts of fructose in their bodies, indicating that wild females do not have to feed on sugar to survive and reproduce (Edman et al. 1992, Van Handel et al. 1994, Costero et al. 1998). *Aedes aegypti* appears to be able to obtain the nutrients necessary for survival and reproduction by feeding frequently on human blood (Scott et al. 1993a, 1993b, 2000a, 2000b; Van Handel et al. 1994). Earlier investigators, who reported greatest egg production by *Ae. aegypti* fed nonhuman blood, were studying mosquitoes that had fed on sugar before a single blood meal (Woke 1937b, Greenberg 1951, Lea et al. 1958, Spielman and Wong 1974, Chang and Judson 1977, Briegel 1985). Although some would argue that there may be differences in the concentrations of sugar in the blood of these hosts, these levels are likely too low to impact mosquito nutrition. Human blood does tend to contain more glucose (≈90 mg/dl [Guyton 1986]) than mouse blood (≈49 mg/dl–60 mg/dl [Alarcon-Aguilar et al. 1997, Sharma et al. 1997]), but the concentrations are low (0.9% for human blood and 0.5–0.6% for mouse blood) compared with an equivalent amount of a 20% sucrose solution; a concentration often used to maintain captive mosquitoes (Foster 1985).

Results from our fourth experiment explained why we had not observed higher egg production by mosquitoes fed nonhuman blood. A sugar meal was required before a single blood meal for the sub-optimal egg production qualities of human blood to become apparent. When sugar feeding was followed by a single high isoleucine blood meal (mouse), egg production was significantly greater than after a low isoleucine blood meal (human). Our results for mosquitoes fed sugar were similar to those of Nayar and Sauerman (1975) who reported greater egg production by *Ae. aegypti* maintained on sugar before ingesting a chick blood meal compared with females provided only water before feeding on a chick.

Our fourth experiment demonstrated that it was important to follow a feeding regime in the laboratory that reflects the diet of wild mosquitoes to avoid study behaviors that are rare for mosquitoes in their natural setting, where they transmit pathogens. In the case of *Ae. aegypti*, that would require feeding them human blood and not sugar.

Our life-table study provided insight into the effects of components of host blood on *Ae. aegypti* fitness. Human-fed females without access to sugar survived almost twice as long as their mouse-fed counterparts. This result is consistent with the prediction that when *Ae. aegypti* are fed high isoleucine blood and not provided a source of carbohydrate, most or all nutrients in the blood meal are used for vitellogenesis, resulting in even frequently fed mosquitoes starving to death due to depletion of maintenance reserves. Although sugar augmented egg production for females fed mouse blood plus sugar, they did not live as long and, therefore, produced fewer total eggs over their lifetime than mosquitoes fed human blood without sugar. We are not aware of evidence indicating that there are species specific differences in host blood that effect egg viability; i.e., hatch rate. We provided water soaked cotton pledgets to mosquitoes fed blood and no sugar and did not compare groups offered blood alone versus blood and water, because of the high water content in blood (Altman and Dittmer 1964). Moreover, wild mosquitoes have access to water at least as often as when they oviposit. Comparison of the measures of fitness we examined (R_o and r) indicated a significant selective advantage for female *Ae. aegypti* fed blood with a low rather than high isoleucine concentration.

We did not detect a difference in survival for mosquitoes offered human blood compared with human blood and sugar, yet those fed human blood and no sugar had significantly greater egg production and relative fitness (R_o). Results from our life-table study (experiment 5) are consistent with the conclusion from earlier investigations that a diet limited to human blood with access to water maximizes *Ae. aegypti* fitness (Scott et al. 1997, Costero et al. 1998b, Morrison et al. 1999, Naksathit and Scott 1998).

In a recent study on the effects of diet on oviposition and survival by *Ae. aegypti*, Canyon et al. (1999) concluded that a sugar and blood diet is likely to occur in nature. Unfortunately, they did not fully consider the evolutionary implications of their study. They did not calculate measures of fitness and only studied females for 12 d. Their data, which indicated greater egg production and no difference in survival for females offered human blood and water compared with a diet of human blood and sugar, conflicted with their conclusion. They provided no new field data to support the idea that sugar feeding is common. Their assumption that host seeking and blood feeding are risky activities is refuted by the high rates of multiple feeding reported for wild *Ae. aegypti* (Scott et al. 1993a, 2000b; Chow-Shaffer et al. 2000) and the lack of a difference between the recapture rates for *Ae. aegypti* in Thailand that were offered only water versus those fed human blood before release (Day et al. 1994).

For *Ae. aegypti*, which do not need to fly far to locate human hosts or oviposition sites (Morland and Hayes 1958, Neff et al. 1967, Trpis and Hausermann 1986, Edman et al. 1998), feeding preferentially and frequently on human blood, and seldom on plant sugar, confers energetic and fitness benefits that in combination constitute a significant selective advantage. From a public health perspective, selection for frequent human contact and an age-dependent increase in feeding frequency magnify the potential for the transmission of pathogens by older potentially in-

fected mosquitoes. We propose that the energetic processes which support the *Ae. aegypti*–human interaction are fundamental to this species' efficiency as a disseminator of human pathogens. An extension of this conclusion is that (1) the population densities of female *Ae. aegypti* necessary to sustain virus transmission will be low because of the tendency of this species to frequently and preferentially bite human hosts (reviewed by Kuno 1997, Focks et al. 2000) and (2) attempts to genetically manipulate *Ae. aegypti* host preference (Curtis 1994) may be difficult due to the fitness advantages associated with a diet that emphasizes the ingestion of human blood.

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