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INCREASED FECUNDITY OF *AEDES AEGYPTI* FED HUMAN BLOOD BEFORE RELEASE IN A MARK–RECAPTURE STUDY IN PUERTO RICO

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ABSTRACT. Laboratory experiments suggest that utilization of blood rather than natural sugar sources for energetic needs affords female *Aedes aegypti* a reproductive advantage over conspecifics that use sugar. To test this hypothesis under field conditions, we carried out a mark–release–recapture study in Florida, PR. Adult females (F_1) reared from field-collected eggs were provided with a diet of human blood alone or human blood plus a 20% honey solution before their release. Backpack aspirators were used to collect mosquitoes from release houses for 5 consecutive days beginning the 2nd day after release. Survival was estimated from the slope of the regression line of the log-transformed daily number of recaptures for each treatment group. To compare fecundity of the treatment groups, each recaptured female was dissected, ovaries were removed, oocytes counted, and Christophers' stages of oocyte development scored. Recapture rates were 30% for the blood-only group and 23% for blood plus honey group. The daily survival rate of the blood-only group (55%) was not statistically different from that of the blood plus honey group (69%) ($t = 0.32$, $P > 0.05$). By analysis of variance, fecundity (average number of stage III–V oocytes) was significantly higher in the females fed human blood alone ($n = 103$, 109 oocytes/female) than in the group fed on blood and honey ($n = 50$, 95 oocytes/female) ($P = 0.0007$). The observed gonotrophic cycle length of the recaptured females ranged from 3 to 7 days. Results from our field study are consistent with laboratory life-table experiments that suggest feeding exclusively on human blood provides a reproductive advantage for female *A. aegypti.*

KEY WORDS *Aedes aegypti,* fecundity, sugar-feeding, gonotrophic cycle, mark–release–recapture, Puerto Rico, survival, fitness

INTRODUCTION

Females of most mosquito species require both blood and sugar to reach their full reproductive potential and support their daily energy needs (Foster 1995). In contrast, female *Aedes aegypti* (L.) seem to have adapted to urban environments where blood is readily available and access to sugar may be limited (Macfie 1915, Foster 1995), and consequently use blood for both their energetic and reproductive requirements (Edman et al. 1992, Van Handel et al. 1994, Scott et al. 1997, Costero et al. 1998b). Physiologically, this adaptive strategy is supported by the observations that *Ae. aegypti* utilize blood for survival at higher rates than do nondomestic mosquitoes (Nayar and Sauerman 1975), take multiple blood meals during a single gonotrophic cycle (Scott et al. 1993a, 1993b), and feed predominantly on protein-rich but isoleucine-limited human blood (Briegel et al. 1990, Chow et al. 1993). The strategy of using human blood rather than plant nectars as an energy source results in an increase in the biting rate of *Ae. aegypti* on humans and consequently confers a selective advantage to the mosquito and to the pathogens that it transmits, such as dengue virus (Scott et al. 1997). Because an increase in a vector's biting rate can exponentially increase transmission of a vector-borne pathogen (Garrett-Jones and Shidrawi 1969, Dye 1992), this blood-feeding strategy may help to explain the observation of explosive dengue epidemics in the absence of large *Ae. aegypti* populations (Gubler 1992).

Recent life-table experiments reported from Thailand (Scott et al. 1997) and Puerto Rico (Costero et al. 1998b) indicated that *Ae. aegypti* fed only human blood have a fitness advantage over females fed both blood and sugar ad libitum. Mosquitoes used in those laboratory experiments emerged from pupae collected at field sites and were maintained in outdoor insectaries at ambient temperatures. The fitness advantage afforded by feeding only on human blood seems to be independent of differences in adult female size and, therefore, teneral energy reserves (Naksathit and Scott 1998). Because the energy requirements of mosquitoes maintained in cages are likely to differ from those living under natural conditions, observations from the previous laboratory-based life table experiments needed to be tested in the field.

The objective of this experiment was to determine, under field conditions in Puerto Rico, whether fitness of female *Ae. aegypti* is higher in those that feed on blood alone as compared to those that feed on sugar and blood. Although it is not possible to duplicate laboratory life-table experiments with free-ranging mosquitoes, we were able to compare the number of developing oocytes and survival based on recaptured females from cohorts fed either blood alone or blood plus sugar before their release.

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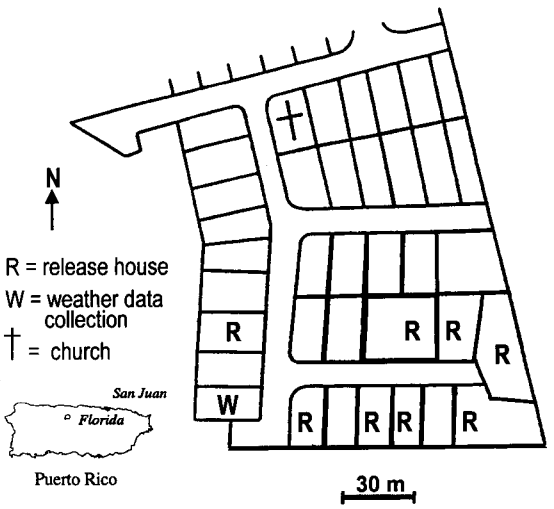

Fig. 1. Map of study area in Yanes III, Florida, PR.

MATERIALS AND METHODS

Study area: Our mark–release–recapture experiment was carried out in a cluster of houses located in a residential neighborhood (Yanes III) in the municipality of Florida, PR. This rural community (26 km^2) is located in the hills of north central Puerto Rico and has a population of 8,689 (U.S. Census Bureau 1990). Endemic dengue virus transmission has occurred there since 1991, when Florida had the highest reported incidence of dengue (15.7 cases per 1,000) in Puerto Rico (Rodríguez-Figueroa et al. 1995). Study houses were situated at the southern end of Yanes III at the base of eroded karst limestone hills, which surrounded study houses on 3 sides. Houses selected for our study had high recapture rates for *Ae. aegypti* in previous mark–release–recapture studies (Edman and Scott, unpublished data).

Temperature and rainfall were recorded at approximately 1600 h each day using a maximum–minimum thermometer and rain gauge. The thermometer and rain gauge were placed in the front yard of a house on the same block as the study houses (Fig. 1).

Mosquitoes: Eggs of *Ae. aegypti* were collected using enhanced ovitraps (Reiter et al. 1991) in Yanes III during January–February 1997, and hatched in the insectary of the Dengue Branch, Centers for Disease Control and Prevention in San Juan, PR, on March 19, 1997. Larvae (200 per enamel pan in approximately 3 cm of water) were reared at ambient temperature (27°C), and fed a standard diet of rabbit chow pellets (NutriMix Feed Company, Cataño, PR) to produce large mosquitoes (Day et al. 1994). Pupae were transferred to 45-cm square screen rearing cages on March 28, where they were allowed to mate, and then transported to our field laboratory in Florida.

To obtain females that fed on blood alone and females that fed on both blood and sugar, we initially separated females into sleeved 4-liter plastic cages with nylon mesh tops. Four of the cages (140 females/cage) were provided cotton pledgets soaked with water; females in the other 4 cages (174 females/cage) were given cotton pledgets soaked with a 20% honey solution for 10 h (1300–2300 h), after which pledgets were removed. The next morning (April 1) each female in the honey treatment was checked under a stereoscopic microscope and those that had enlarged crops were placed in separate 4-liter cages from those in which sugar feeding could not be verified (abdomen flat). Each group (water only and honey only) was then offered the opportunity to bloodfeed from the arm of either A.C. or A.C.M. at 1300 h (for 10 min) and again at 1540 h (for 5 min). Bloodfeeding was confirmed by visual examination. For the remainder of the manuscript we will refer to the following 2 treatment groups. Blood only: females fed a diet of water and human blood before release. Blood plus honey: females with enlarged crops from the cage provided honey-soaked pledgets and fed human blood before release. Because the sugar-feeding status of the remaining females (with flat abdomens) from the cage provided honey-soaked pledgets could not be determined, we will not present results from this group.

After the 1st blood meal, engorged females from the 2 treatment groups (blood only, blood plus honey) were placed in separate labeled cardboard cages (1 pint) for each of the 8 release houses (16 containers total). The blood plus honey group was provided honey-soaked pledgets before release, whereas the blood-only group was provided water-soaked cotton pledgets until release.

Marking: The blood-only and blood plus honey release groups were marked with orange and green fluorescent powder (Dayglo Color Corp., Cleveland, OH), respectively. To dust the mosquitoes, nylon mesh lids of the cardboard cages were covered with clear plastic wrap, fluorescent powder was placed in a 5-cc disposable syringe, the tip of the syringe was pushed through a small hole at the base of the cage, and the plunger was depressed with force. This created a fine cloud of fluorescent powder inside the container that settled on the mosquitoes. The containers were examined under a stereoscopic microscope to verify that the females had been marked.

Release: In each of 8 release houses, we attempted to release 50 females from the blood only and 30 females from the blood plus honey groups in a central hallway (640 total) between 1800 and 1830 h on April 1, 1997 (Fig. 1). Females that did not voluntarily fly out from their carton when the lid was removed and the carton tapped were not included in the study. At the time of release, all mosquitoes from each group were confirmed to have taken a blood meal.

Recapture: After allowing 1 day for the released mosquitoes to disperse, battery-powered backpack aspirators (John C. Hock, Gainesville, FL) were used to recapture mosquitoes for 5 consecutive days from inside each of the 8 release houses. This highly effective method captures all physiologic stages of resting mosquitoes within a brief period of time, ca. 10–15 min (Edman et al. 1992; Scott et al. 1993a, 1993b; Clark et al. 1994; Van Handel et al. 1994). Captured mosquitoes were anesthetized with carbon dioxide and then placed in plastic tubes on wet ice for at least 30 min. Each female *Ae. aegypti* was examined carefully under a stereoscopic microscope at 40× for fluorescent dust markings. The number of marked and wild *Ae. aegypti* was recorded daily. All recaptured mosquitoes were kept on wet ice and dissected on the same day as they were collected.

Mosquito dissections: The physiologic status of each recaptured female was recorded. The following categories were used: empty (flat abdomen without blood), fully engorged (abdomen with <¼ developed eggs and a fresh, red blood meal), half gravid (old digested blood meal plus developing eggs composing ¼–¾ of the abdomen), and gravid (abdomen >¾ full of eggs, with or without a dark tar spot or small fresh blood meal). In addition, it was noted when gravid, some females with stage V oocytes had a flat appearance.

Recaptured females were dissected in a drop of 10% phosphate-buffered saline (PBS). Ovaries were removed from the abdomen, placed in another drop of PBS, and ovarioles were separated using dissecting needles. The number and Christophers' stage (I–V) of all the oocytes were recorded for each ovary (Christophers 1960). Because it was difficult to separate individual oocytes in immature ovaries without damaging them, we did not attempt to count stage I oocytes.

Data analysis: Recapture rates among the release groups, collection houses, and recapture days were compared by chi-square analyses using PROC FREQ in SAS (SAS Institute 1989). Chi-square analysis was also used to compare the physiologic status of recaptured females; for these comparisons categories were pooled into gravid and not gravid (empty, engorged, or half-gravid) to comply with the assumptions of the text. To estimate and compare daily survival rates, regression lines of log-transformed daily recaptures were generated for each cohort and slopes of the lines were compared using a *t*-test following the methods of Zar (1984). Average daily survivorship estimates were generated for each cohort by calculating the antilog of the regression coefficient for each age group (Gillies 1961). Because eggs do not develop past Christophers' stage II without ingestion of a blood meal (Christophers 1960, Clements and Boocock 1984), oocytes ≥ stage III were considered the most likely to represent the total number of viable eggs that would have been oviposited if the mosquito had not been recaptured. Thus, to compare fecundity of the 2 treatment groups we used analysis of variance (ANOVA) to test for significant differences in the mean number of stage III–V oocytes per female among the treatment groups, physiologic status categories, and recapture days. This was done using general linear models (PROC GLM) of SAS (SAS Institute 1989). The number of oocytes for each recaptured female was log-transformed to reduce hetereoscedasticity and ensure a normal distribution of residuals. Full-rank ANOVA models including all interaction terms were developed and then nonsignificant terms ($P > 0.05$) were removed in a stepwise fashion until the most parsimonious model was achieved. Tukey's honestly significant difference (HSD) was used to test for differences among treatment groups.

RESULTS

A total of 391 female *Ae. aegypti* from the blood-only group and 235 females from the blood and honey group were marked and released. The recapture rate of females fed only blood before release (30%) was similar to the recapture rate for the blood and honey group (23%; $\chi^2 = 3.13$, df = 1, $P = 0.077$).

Aedes aegypti from each treatment group were recovered on each of the 5 collection days. The number of recaptured females declined over the collection period. The recapture rate of each treatment group was independent of collection day ($\chi^2 = 4.1$, df = 4, $P = 0.395$; Fig. 2). Likewise, recapture rates in each of the collection houses ranged from 15 to 41% but the proportion of each treatment group recaptured was consistent among all of the houses ($\chi^2 = 8.6$, df = 7, $P = 0.277$; Table 1).

Maximum and minimum daily temperatures during the experimental period (April 1–7) averaged (±SD) 27 ± 2°C and 19 ± 2°C, respectively. Daily rainfall ranged from 0 to 31 mm. In general, temperature was highest on April 1 (release day) and lowest on April 3 (1st recapture day) when the highest daily rainfall was recorded. Temperatures then increased slowly for the remainder of the experimental period.

Survival

Daily survival rates estimated from the exponential model were 55% for the blood-only ($n = 117$) and 69% for the blood plus honey ($n = 55$) groups (Fig. 3); however, these rates were not statistically different ($t = 0.32$, $P > 0.05$).

Fecundity

We dissected 164 of the 172 recaptured *Ae. aegypti* (95%). Nine of those individuals had ovaries containing oocytes at stage I–II. The remaining 155 mosquitoes had oocytes at stages III–V and were used to compare fecundity of the 2 treatment

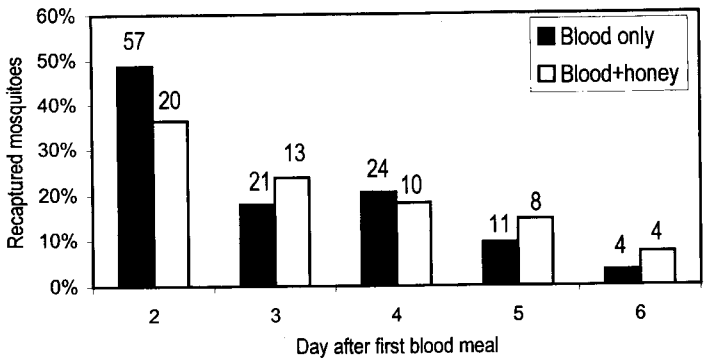

Fig. 2. Percentage of female *Aedes aegypti* fed blood alone or blood and honey before release that were recaptured each day between April 3 and 7, 1997. Above each bar is the number of the females recaptured. The day on the x axis represents the number of days after the mosquitoes were bloodfed and released on April 1, 1997.

groups by ANOVA. In 91% of the recaptured females, the stage of oocytes was uniform; the remaining 9% contained oocytes in different stages of development. On average, blood-only group produced more oocytes (109 oocytes/mosquito) than the blood and honey group (95 oocytes/mosquito; Tukey's HSD, $P < 0.05$; group Table 2).

Physiologic status

Both the physiologic status ($\chi^2 = 79$, df = 4, $P = 0.001$; gravid vs. other) and oocyte development ($\chi^2 = 73$, df = 4, $P = 0.001$; stage V vs. other) of the recaptured females varied with recapture day. This variation was consistent with a 3 to 7-day gonotrophic cycle (defined here as the time interval between blood feeding and completion of oviposition). Of the 164 mosquitoes that were dissected, only 4 (2%) were empty and 3 (2%) were fully engorged. On the 1st recapture day, 2 days after their 1st blood meal, 97% (69 of 71) of the recaptured mosquitoes contained a partially digested blood meal and developing eggs (Figs. 4A, 4B) and 85% (60 of 79) had stage III–IV oocytes (Figs. 4C, 4D). During the following 2 days most of the recaptured females were gravid (73%; Figs. 4A, 4B) and had stage V oocytes (89%; Figs. 4C, 4D). Five to 6 days after bloodfeeding and release, about 50% of the recaptured females showed evidence of starting (half-gravid and empty) their 2nd gonotrophic cycle (Fig. 4).

Treatment group differences in the physiologic status and oocyte development of the recaptured females over time could not be confirmed statistically. However, based on when empty females with stage I–II oocytes were 1st recaptured, the length of the gonotrophic cycle seemed to be shorter in the blood only group (Figs. 4A, 4C). Empty females were 1st observed 3–4 days after their 1st blood meal in the blood-only group and after 5–6 days in the blood plus honey group.

Table 1. Summary of marked *Aedes aegypti* recaptured inside 8 release houses in April 1997 in Florida, PR.

House no.	No. recaptured/no. released (% recapture)		
	Blood only	Blood plus honey	Total
1	14/47 (30)	7/30 (23)	21/77 (27)
2	13/49 (27)	9/30 (33)	22/79 (27)
3	26/46 (57)	14/27 (52)	30/73 (41)
4	9/50 (18)	2/30 (7)	11/80 (14)
5	12/50 (24)	0/30 (0)	12/80 (15)
6	15/50 (30)	6/30 (20)	21/80 (26)
7	16/50 (32)	11/29 (41)	27/79 (34)
8	12/49 (25)	6/29 (17)	18/78 (23)
Total	117/391 (30)	55/235 (23)	172/626 (27)

DISCUSSION

Early life-table studies of *Ae. aegypti* were carried out under laboratory conditions, exposed females to sugar before bloodfeeding, used nonhuman blood sources, and limited access of females to blood (Crovello and Hacker 1972, Lansdowne and Hacker 1975, Day et al. 1994). Recent life-table experiments (Scott et al. 1997, Costero et al. 1998b, Naksathit and Scott 1998) have more effectively approximated natural conditions where this species feeds frequently on human blood (Chow et al. 1993; Scott et al. 1993a, 1993b) and infrequently on sugar (Edman et al. 1992, Van Handel et al. 1994, Costero et al. 1998a). These recent studies compared the net reproductive rate (R_o) and intrinsic rate of growth (r) of cohorts of mosquitoes offered diets of human blood or human blood plus sugar.

Our study complements these previous life-table experiments by comparing fecundity, survival, and gonotrophic cycle length of similar cohorts of female *Ae. aegypti* under field conditions. Although we could not calculate R_o (Σdaily survival $\times$ eggs laid/female/day) or r ($\ln R_o$/mean generation time,

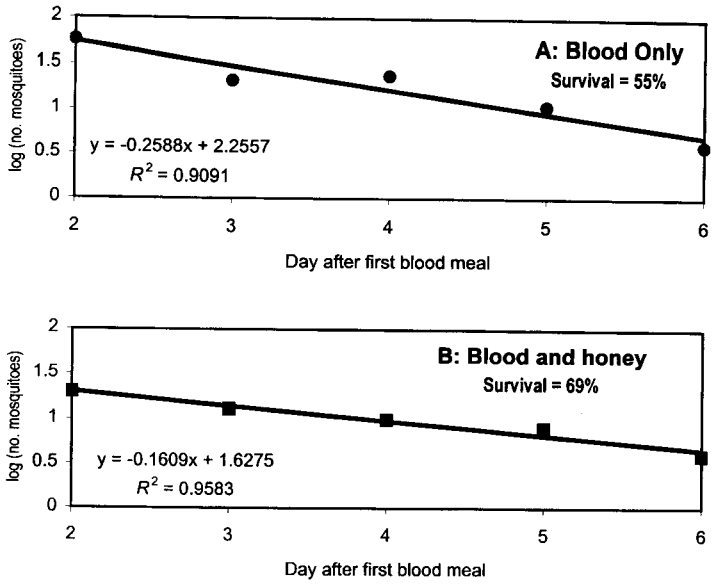

Fig. 3. Regression lines for the number of recaptured mosquitoes (log-transformed) by recaptured day for each treatment group. The day on the x axis represents the number of days after the mosquitoes were bloodfed and released on April 1, 1997. (A) Blood only. (B) Blood plus honey.

potential for population expansion) directly, survival and fecundity estimates in our study are surrogates for R_o and gonotrophic cycle length is a component of r. Thus, we can make indirect comparisons of fitness between the treatment groups. Because fecundity was estimated for a single gonotrophic cycle, and daily probability of survival for a 5-day period, rather than over the entire lifetime of the mosquitoes, estimates of fecundity, survival, and gonotrophic cycle length should be viewed in relative rather than in absolute terms.

Our results are consistent with the earlier life-table studies in Puerto Rico that reported higher age-specific survival rates in female mosquitoes fed a diet of human blood plus sugar but significantly higher fecundity in mosquitoes fed blood alone (Costero et al. 1998b). In contrast, in Thailand age-specific survival rates were highest in the blood-only group, whereas no significant fecundity differences were found among the 2 diet cohorts (Scott et al. 1997). Our results showed a clear fecundity advantage in the females fed a diet of blood alone, whereas we were unable to detect a statistically significant difference in the daily probability of survival between the 2 treatment groups. However, further studies with larger sample sizes or replicates are necessary to more definitively address this issue of survival.

Table 2. Mean number of oocytes (±SE) for all *Aedes aegypti* recaptured between April 3 and 7, 1997, in Florida, PR.

	Treatment group	
No. oocytes	Blood only	Blood plus honey
n	103	50
Mean ± SE	109 ± 2[1]	95 ± 4

[1] Mean no. eggs in blood-only group significantly greater than in both sugar groups by Tukey's honestly significant difference ($P < 0.05$). Analysis of variance model: log(no. eggs) = treatment group, $R^2 = 0.07$, df = 1, 154, $F = 11.9$, $P = 0.0007$.

Physiologically, the fecundity advantage observed for females in the blood-only group may have been effected by their ability to imbibe larger blood meals than females that had fed on sugar. The initiation of vitellogenesis and egg maturation requires a critical minimum level of blood that is dependent on available energy reserves (Foster 1995). Above this critical minimum, egg production increases with the quantity of blood ingested (Briegel 1985, 1990). Sugar feeding reduces blood meal size, both while sugar is in the crop and after it is converted to energy reserves (Foster et al. 1989). Sugar feeding also reduces host-seeking activity (Klowden 1986), which could limit additional bloodfeeding that would enhance fecundity.

Another important component of fitness is the speed with which *Ae. aegypti* complete their gonotrophic cycle (i.e., generation time). A female with a short egg laying cycle will have a higher probability of laying more egg batches in her lifetime than a female with a longer cycle. Generation time is an integral part of the intrinsic rate of growth. Females in their 2nd gonotrophic cycle were 1st observed in the blood-only treatment group. Similar individuals in the blood plus honey group were not observed until 2 days later. This suggests that in

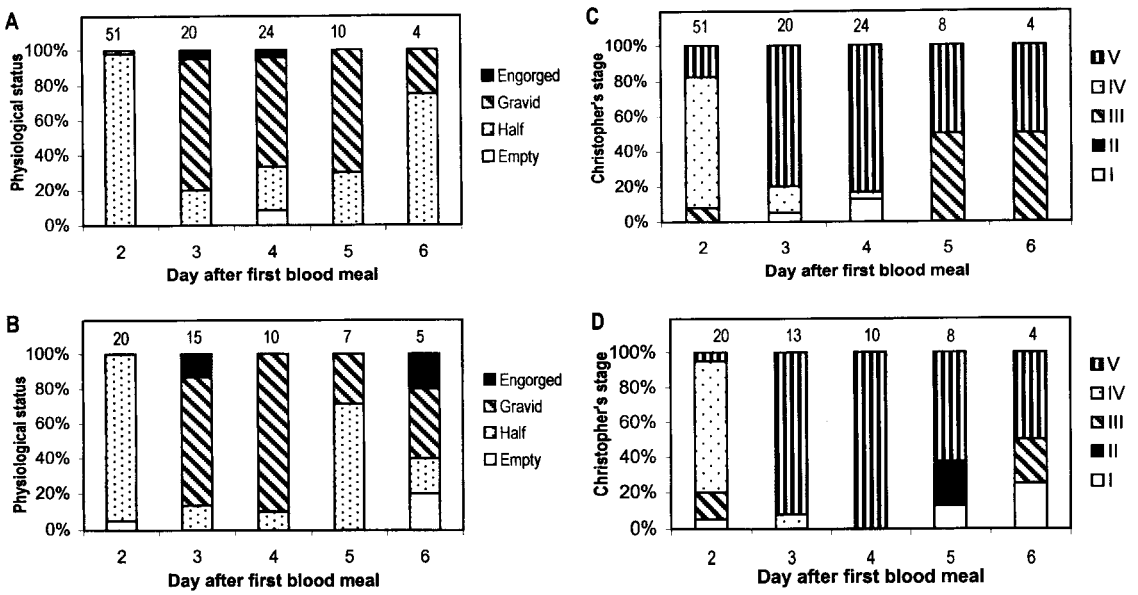

Fig. 4. Characterization of gonotrophic cycle of female *Aedes aegypti* recaptured April 3–7, 1997. The day on the *x* axis represents the number of days after the mosquitoes were bloodfed and released on April 1, 1997. (A) Percentage of recaptured females fed blood alone before release in 4 physiologic status categories by recapture day. (B) Percentage of recaptured females fed blood and honey before release in 4 physiologic status categories by recapture day. (C) Percentage of recaptured females fed blood alone before release with Christophers' stage I–V oocytes by recapture day. (D) Percentage of recaptured females fed blood and honey before release with Christophers' stage I–V oocytes by recapture day.

addition to producing more eggs per gonotrophic cycle the blood-only group produced their eggs more rapidly.

A limitation of our study was that we were not able to determine the sugar-feeding status of mosquitoes after release. However, because sugar feeding seems to be rare in natural *Ae. aegypti* populations (Edman et al. 1992, Van Handel et al. 1994, Costero et al. 1998a) and trends in survival and reproduction were similar to those reported for well-controlled laboratory studies (Scott et al. 1997, Costero et al. 1998b, Naksahit and Scott 1998), the prerelease diet seems to be a good indicator of overall diet. In previous studies carried out in Puerto Rico, natural fructose feeding rates for females ranged from 1% (Van Handel et al. 1994) to 13% (Costero et al. 1998a) depending on the season of collection. Fructose feeding rates in both studies varied significantly with the month in which females were collected. In a study carried out in Chiapas, Mexico, sugar-feeding rates in females varied between 8 and 21% in direct relation to abundance of blooming plants (Martinez-Ibarra et al. 1997), whereas in Thailand, sugar-feeding rates were 4 and 2% during the dry and wet season, respectively (Edman et al. 1992).

Our results provide field support for the hypothesis that the lower rates of sugar feeding observed in female *Ae. aegypti,* compared with males and other mosquito species, are due to the fitness advantage conferred by feeding primarily, or solely, on human blood. In our study, the cohort fed human blood alone before release had the highest measure of fecundity and shortest gonotrophic cycle, suggesting that its R_o and r were higher than in cohorts that fed on sugar. The epidemiologic relevance of this fitness advantage is probably related to the effects of bloodfeeding frequency on virus transmission. Natural variation in blood- and sugar-feeding frequency behavior should be examined under additional environmental conditions and in different geographic locations.

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