

Superior reproductive success on human blood without sugar is not limited to highly anthropophilic mosquito species

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Abstract. Anthropophilic mosquitoes such as *Aedes aegypti* L. (Diptera: Culicidae) have been shown to have superior reproductive success on human blood when sugar is not available. Life-table experiments were conducted with *Aedes albopictus* Skuse and *Ae. aegypti* to compare the effects of sugar availability on age-specific survivorship, lifetime and daily fecundity, and blood-feeding frequency when offered human blood daily. There were no significant interactions between the effects of sugar availability and mosquito species for these four variables, indicating similar effects of sugar availability for both species. Lifetime fecundity was not significantly affected by sugar availability, but sugar-deprived females had significantly reduced age-specific survivorship than did sugar-fed females. In absence of sugar, females took bloodmeals twice as often, resulting in a higher daily fecundity. The results indicate that superior reproductive success on human blood without sugar does not seem to be limited to highly anthropophilic mosquito species, such as *Ae. aegypti*. We conclude that evolution of a highly anthropophilic feeding strategy is not an inevitable result of the ability to thrive on human blood alone.

Key words. *Aedes albopictus*, *Aedes aegypti*, blood-feeding frequency, fecundity, life tables, mosquito, net reproductive rate, sugar-feeding, survivorship.

Introduction

Females of most mosquito species require both blood and sugar for reproduction and survival (Foster, 1995). However, anthropophilic species such as *Aedes aegypti* L. and *Anopheles gambiae sensu stricto* Giles may derive all adult energy requirements from human blood alone (Straif & Beier, 1996; Gary & Foster, 2001; Harrington *et al.*, 2001b). Field studies in domestic environments in Thailand and Puerto Rico showed that female *Ae. aegypti* fed predominantly on humans (Chow *et al.*, 1993; Scott *et al.*, 1993b; Costero *et al.*, 1998), seldom fed on plant sugars (Edman *et al.*, 1992) and blood-fed multiple times within a gonotrophic cycle (Scott *et al.*, 1993a). Similar behaviour has been demonstrated for *An. gambiae s.s.* and *Anopheles funestus* Giles in nature (Beier, 1996). Laboratory feeding studies

suggest that *Ae. aegypti* females fed on blood alone have a reproductive advantage over those offered both human blood and sugar (Scott *et al.*, 1997; Costero *et al.*, 1998; Harrington *et al.*, 2001b). *Aedes aegypti* fed on human blood without access to sugar have higher lifetime fecundity than do individuals fed on human blood with sugar or on non-human vertebrate blood without sugar (Harrington *et al.*, 2001b). Female *An. gambiae s.s.* do not forfeit reproductive fitness if sugar is replaced by increased human blood feeding (Gary & Foster, 2001). Foster & Eischen (1987) demonstrated that sugar deprivation causes a substantial increase in human blood-feeding frequency in *Ae. aegypti*, but not in opportunistic *Anopheles quadrimaculatus* Say. Harrington *et al.* (2001b) suggested that selective and frequent feeding on humans coupled with infrequent feeding on sugars is an adaptation associated with a highly

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domesticated lifestyle adopted by only a few mosquito species. By contrast to these well-studied anthropophilic species, information on the nutritional effects of sugar on survival and fecundity of non-anthropophilic species is limited. Hence, it remains unclear whether the ability to thrive on human blood alone is a characteristic of anthropophilic species, and whether that ability represents a key adaptation to an anthropophilic feeding strategy.

The Asian tiger mosquito, *Aedes albopictus* Skuse, is both a member of the same subgenus as *Ae. aegypti*, and ecologically similar to *Ae. aegypti* in several ways. Like *Ae. aegypti*, *Ae. albopictus* feeds on humans, develops as a larva in artificial containers and is commonly found near houses. In nature, *Ae. albopictus*, however, takes blood from a broader range of vertebrate hosts than does *Ae. aegypti* (Tempelis, 1975; Niebylski *et al.*, 1994; Gomes *et al.*, 2003; Almeida *et al.*, 2005). Local habitat segregation between *Ae. albopictus* and *Ae. aegypti* has been well documented. In zones of sympatry, *Ae. albopictus* is more common in rural areas, *Ae. aegypti* predominates in urban areas and the two species overlap in suburban areas (Hawley, 1988; Braks *et al.*, 2003). Interspecific differences in egg (Juliano *et al.*, 2002) and larval (Juliano, 1998; Daugherty *et al.*, 2000; Braks *et al.*, 2004) characteristics of *Ae. aegypti* and *Ae. albopictus* have failed to explain this local spatial segregation (Juliano *et al.*, 2004). Habitat characteristics, such as human population density and vegetation cover, are likely to affect the ecology of adult mosquitoes. Local habitat differences affect frequency of sugar feeding of mosquitoes (Edman *et al.*, 1992; Van Handel *et al.*, 1994; Martinez-Ibarra *et al.*, 1996; Costero *et al.*, 1998; Gary & Foster, 2004).

In this study, we compare the reproductive success of *Ae. albopictus* and *Ae. aegypti* and test the hypothesis that the effects of sugar availability on survivorship, human blood-feeding frequency, and daily and lifetime fecundity differ between the species. Such differences are predicted if the ability to thrive without sugar is an adaptation to an anthropophilic lifestyle (like that of *Ae. aegypti*) and not necessary for a more generalist lifestyle (like that of *Ae. albopictus*).

Methods and methods

Mosquitoes

Laboratory colonies of *Ae. aegypti* and *Ae. albopictus* were established from larvae and pupae collected from field sites in South Florida in 2002. Adults were kept in cages in an insectary at 25.00 (± 0.04 SE) °C and 84.4 (± 0.8 SE) % RH at 16 : 8 h LD photoperiod and had continuous access to 10% sucrose solution. Females were regularly fed on live chickens and laid eggs on paper towels in water-containing cups. Eggs were hatched in water. Larvae were reared at low densities (50 larvae per litre) in 5 × 20 × 30 cm enamel trays and were fed sufficiently (25 mg yeast/liver powder, 1 : 1, per tray/day) to produce large, similarly sized adults.

Experimental set up

Within 24 h after pupation, one female and two male pupae were placed together in a water-containing cup (20 mL) held in a cylindrical plastic container (400 mL) with a transparent plastic top. An oviposition substrate, consisting of germination paper inserted in a white plastic film canister (3 cm diameter, 4 cm height) half-filled with water, was provided. After a female laid her first egg batch, males were removed from the container. Females of each species were divided into two experimental groups: daily access to human blood with (i) or without (ii) 10% sugar solution, which was offered in a small glass vial (10 mL) with a cotton wick. Each experimental group consisted of 11 female mosquitoes. Beginning 3 days after emergence, each female was given a daily opportunity to feed to repletion. Females were given 10 min to commence feeding on a human arm (volunteer M.B.) through a mesh-covered round opening (20 cm²) in the vertical surface of the plastic container. Each day, oviposition papers with eggs were replaced, and survivorship, blood-feeding success (positive or negative), and the number of eggs were recorded. At death, females were dissected and the numbers of mature eggs (Christophers' stages 4 and 5; Christophers, 1960) in the ovaries were counted. The experiment continued until all mosquitoes had died. At death, the wings of the majority of mosquitoes were worn and unsuitable for size estimates. Two female *Ae. albopictus* of the sugar-fed group, which did not oviposit, were excluded from analyses.

To compare survivorship in experimental treatments in which females received no nutrients, a parallel group was created by placing a female and a male pupa together in a water-containing cup (20 mL) held in a 400 mL plastic cage with access to water only. The number of days alive, an estimate of energy reserves upon emergence, was recorded for each female. At death of a starved female, one of her wings was removed and measured under a dissecting microscope with an ocular micrometer following the methods of Packer & Corbet (1989). There were 20 and 19 replicates for *Ae. aegypti* and *Ae. albopictus*, respectively, in these starvation treatments.

Statistical analyses

For each female, longevity, daily and lifetime fecundity, and blood-feeding frequency were determined. Longevity was determined as the number of days from the first blood-meal to death. Lifetime fecundity was measured as the total number of eggs matured during a female's lifetime and daily fecundity (number of eggs per day) as the total number of eggs divided by longevity. Blood-feeding frequency was calculated as the total number of bloodmeals divided by longevity, which is equivalent to the proportion of days on which the female took blood. These proportions were arcsine, square-root transformed before analyses. Daily and lifetime fecundity and blood-feeding frequency were analysed using least squares two-way factorial ANOVA (PROC GLM; SAS Institute Inc., 1989) with species

(*Ae. aegypti* vs. *Ae. albopictus*), and sugar availability (sugar vs. no sugar) as factors. Age-specific survivorship of females was compared among treatments and species using non-parametric survival analysis (PROC LIFETEST, SAS Institute Inc., 1989).

Wing lengths of starved females were compared between species using a Student's *t*-test. Age-specific survivorship of starved females was compared between species using non-parametric survival analysis (PROC LIFETEST, SAS Institute Inc., 1989).

Results

Effects of sugar

The age-specific survivorship (l_x , x = age in days) was significantly affected by treatment ($\chi^2 = 7.86$, d.f. = 1, $P = 0.049$), but not by species ($\chi^2 = 2.83$, d.f. = 1, $P = 0.092$). Survival of sugar-fed females was significantly greater than that of sugar-deprived females (Fig. 1A). *Post hoc* comparisons of means between sugar treatments within species were not significantly different ($P > 0.05$). Data on the expected number of daughters (m_x = mean number of eggs/2 for each age x), net replacement rate ($R_0 = \sum l_x m_x$), and cumulative number of bloodmeals are shown in Fig. 1 for qualitative evaluation. For both species, sugar-deprived females tended to have greater m_x (Fig. 1B), and greater R_0 (Fig. 1C) early in their adult lives (days 10–50) than did sugar-fed females. These values were approximately equal by the end of the experiment (Figs 1B and C).

Access to sugar yielded significantly lower daily fecundity and blood-feeding frequency (Tables 1 and 2). There were no significant interactions between the effects of mosquito species and treatment, indicating that the effects of sugar availability on these variables were similar for both species (Table 2). Life-time fecundity was not significantly affected by sugar availability but was affected by species (Table 2). *Aedes aegypti* produced more eggs than did *Ae. albopictus* (Table 1). There were no significant differences between the two mosquito species for blood-feeding frequency or daily fecundity (Table 2).

Unfed females: size and age-specific survivorship

For the water-only treatment group, wings of female *Ae. aegypti* (3.00 mm \pm 0.02 SE) were significantly longer than those of *Ae. albopictus* (2.90 mm \pm 0.01 SE) (Student's *t*-test, d.f. = 38, $t = 3.34$, $P = 0.002$). Age-specific survivorship of starved females was not significantly affected by species ($\chi^2 = 0.56$, d.f. = 1, $P = 0.455$).

Discussion

Our results show that females of both *Ae. aegypti* and *Ae. albopictus* were capable of surviving on human blood

without sugar. This, together with the fact that females maintained on human blood alone had increased age-specific survivorship compared to those on water alone, demonstrates that females of both species can use at least part of the bloodmeal for their energy requirements, confirming previous laboratory studies on female *Ae. aegypti* (Briegel, 1985; Day *et al.*, 1994; Scott *et al.*, 1997; Harrington *et al.*, 2001b). Sugar availability significantly affected daily but not lifetime fecundity of females of both species. Females maintained on human blood alone laid as many eggs during their lifetimes as did females with access to blood and sugar, despite the fact that the latter group had greater longevity. This is consistent with reports for female *Ae. aegypti* blood-fed on chickens (Day *et al.*, 1994), but not for those fed on humans, which have been reported to have higher R_0 when fed on blood alone (Scott *et al.*, 1997; Harrington *et al.*, 2001b). In the absence of sugar, both *Ae. aegypti* and *Ae. albopictus* females fed on blood about twice as often as when sugar was available, which is consistent with previous laboratory reports on *Ae. aegypti* on the effect of sugar on host seeking behaviour (Klowden, 1987) and blood-feeding frequencies (Foster & Eischen, 1987; Canyon *et al.*, 1999). Although we did not determine or control the amount of blood ingested, we presume that the increased daily fecundity was caused by an increased daily blood intake during sugar deprivation, despite the fact that part of the bloodmeal was used for maternal maintenance (see above). Our data show that R_0 for both species was initially higher for females fed on human blood alone than for those with access to sugar, but by the end of the experiment this difference had disappeared.

In previous studies, the survival of female *Ae. aegypti* fed on human blood was much lower than that observed in our study, with median survival times (LT₅₀) of 18 and 20 days (Scott *et al.*, 1997) and 14 and 14.5 days (Harrington *et al.*, 2001b) when fed on human blood with and without sugar, respectively, as opposed to 53 and 25 days (Fig. 1a) in our study. The low median survival times reported by Scott *et al.* (1997) may have been due to their use of females that were collected from the field as pupae, and so were most likely not optimally fed as larvae.

Our data suggest that females of both species reached their full reproductive potential when feeding on human blood under optimal laboratory conditions, regardless of sugar availability. The significant difference between the fecundity of the two species is likely associated with the larger size of *Ae. aegypti*. Positive correlations between mosquito size and fecundity are well established (Briegel, 1985; Lounibos *et al.*, 2002).

For both species, fecundity early in life is greater without sugar feeding. From an evolutionary perspective, high fecundity early in life is advantageous, given the low daily survival probability of adult *Ae. aegypti* in the field, ranging from 0.73 (Harrington *et al.*, 2001a) to 0.93 (Costero *et al.*, 1998). As noted above, longevity in the laboratory of females collected from the field as pupae appears to be considerably less than that of females reared under the near-ideal conditions in our experiment. Thus, the

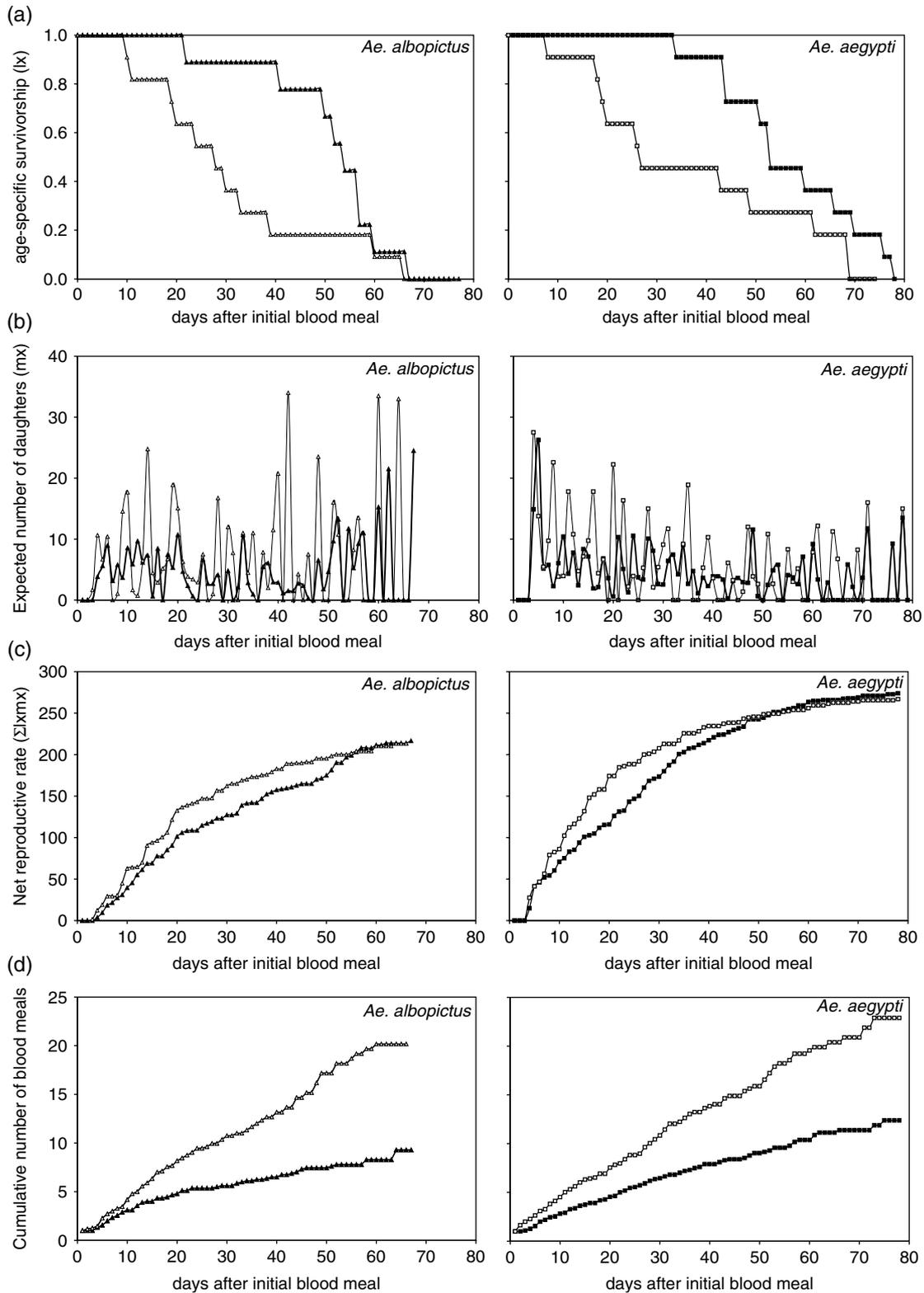


Fig. 1. Life table results for *Aedes albopictus* (left) and *Ae. aegypti* (right) allowed to feed on a human host daily with (solid symbol) or without (open symbol) access to sugar. (A) Age specific survival (l_x). (B) Expected number of daughters (m_x). (C) Cumulative net reproductive rate ($R_0 = \sum l_x m_x$). (D) Cumulative number of bloodmeals.

Table 1. Means (\pm SE) of longevity, lifetime fecundity, daily fecundity and blood-feeding frequency for the mosquito cohorts fed on humans.

Species	Treatment	Longevity (days)	Lifetime fecundity (eggs)	Daily fecundity (eggs/day)	Blood-feeding frequency (meals/day)
<i>Aedes aegypti</i>	Sugar	57.18 (4.26)	547.91 (31.60)	9.86 (0.65)	0.18 (0.02)
	No sugar	38.09 (7.09)	533.73 (73.04)	16.14 (1.54)	0.35 (0.03)
<i>Aedes albopictus</i>	Sugar	51.11 (4.35)	378.22 (57.70)	7.56 (0.97)	0.15 (0.02)
	No sugar	30.91 (5.48)	426.73 (88.74)	13.43 (1.48)	0.34 (0.03)

Table 2. Least squares two-way ANOVA results of the three variables for the mosquito cohorts with species and treatment as factors (see Table 1). Effects significant at $P = 0.05$ are highlighted in bold.

Source	d.f.	Lifetime fecundity		Daily fecundity		Blood-feeding frequency	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Species	1	4.20	0.0474	4.03	0.051	0.65	0.4244
Treatment	1	0.06	0.8000	23.71	< 0.0001	43.07	< 0.0001
Species*treatment	1	0.22	0.6452	0.03	0.865	0.25	0.6165
Error	38						

advantage of higher early fecundity in the absence of sugar may be less apparent in our laboratory experiment than it would be in the field, because of the greater longevities of individuals reared under optimal conditions in the laboratory, as opposed to suboptimal nutritional conditions in the field.

In conclusion, our results support Scott *et al.* (1997), in that feeding by *Ae. aegypti* on human blood alone results in superior reproductive success compared to feeding on human blood and sugar, particularly early in female life. Contrary to the interpretation of Scott *et al.* (1997) that this fitness benefit is associated with the highly anthropophilic habit of *Ae. aegypti*, our results show that this benefit is present for both *Ae. aegypti* and the less anthropophilic *Ae. albopictus*. These results do not support the hypothesis that there are species-specific differences in the effect of sugar availability on life tables of *Ae. albopictus* and *Ae. aegypti* when fed on human blood. The advantage of anthropophilic feeding might result from nutritional properties of human blood, rather than from a metabolism unique to the mosquito species. Human blood has low levels of isoleucine compared to other vertebrates, which limits egg production in general (Briegel, 1985). This means that other amino acids are available in excess of what can be used for eggs and available for other metabolic uses. Therefore, in contrast to non-human blood, larger proportions of human blood can be converted into energy for maternal maintenance (Briegel, 1985, 1990a,b) in the absence of sugar feeding. We hypothesize that the ability to thrive on human blood alone is not the defining feature inevitably associated with the highly anthropophilic feeding strategy and peridomestic lifestyle of species like *Ae. aegypti*. Possible additional adaptations necessary for the anthropophilic lifestyle include: (1) species-specific innate behavioural differences leading to host preferences; (2) species-specific fitness differences when fed on non-human blood (Klowden & Chambers, 1992); or (3) differential tolerances of environmental conditions associated with human-dominated habitats.

An alternative hypothesis is that the closely related *Ae. aegypti* and *Ae. albopictus* share the ability to thrive on an exclusively human-blood diet, but that other species do not, and that *Ae. albopictus* is the more flexible of the two species. Yee & Foster (1992) showed that these two members of the subgenus *Stegomyia* take an unusual high priority in blood feeding over sugar feeding when compared with three other species. Future studies, including one or more zoophilic mosquito species and alternative (non-human) blood sources, are needed to reach a more general conclusion.

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References

- Almeida, A.P.G., Baptista, S.S.S.G., Sousa, C.A.G.C.C. *et al.* (2005) Bioecology and vectorial capacity of *Aedes albopictus* (Diptera: Culicidae) in Macao, China, in relation to dengue virus transmission. *Journal of Medical Entomology*, **42**, 419–428.
- Beier, J.C. (1996) Frequent blood-feeding and restricted sugar feeding behavior enhance the malaria vector potential of *Anopheles gambiae s.l.* & *An. funestus* (Diptera: Culicidae) in western Kenya. *Journal of Medical Entomology*, **33**, 613–618.
- Braks, M.A.H., Honório, N.A., Lourenço-de-Oliveira, R., Juliano, S.A. & Lounibos, L.P. (2003) Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in

- southeastern Brazil and Florida, USA. *Journal of Medical Entomology*, **40**, 785–794.
- Braks, M.A.H., Honório, N.A., Lounibos, L.P., Lourenço-de-Oliveira, R. & Juliano, S.A. (2004) Interspecific competition between two invasive species of container mosquitoes *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazil. *Annals of the Entomological Society of America*, **97**, 130–139.
- Briegel, H. (1985) Mosquito reproduction: incomplete utilization of the blood meal protein for oögenesis. *Journal of Insect Physiology*, **31**, 15–21.
- Briegel, H. (1990a) Metabolic relationship between female body size and reserves and fecundity of *Aedes aegypti*. *Journal of Insect Physiology*, **36**, 165–172.
- Briegel, H. (1990b) Fecundity, metabolism, and body size in *Anopheles* (Diptera, Culicidae), vectors of malaria. *Journal of Medical Entomology*, **27**, 839–850.
- Canyon, D.V., Hii, J.L.K. & Muller, R. (1999) Effect of diet on biting, oviposition, and survival of *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, **3**, 301–308.
- Chow, E., Wirtz, R.A. & Scott, T.W. (1993) Identification of blood meals in *Aedes aegypti* by antibody sandwich enzyme-linked immunosorbent assay. *Journal of the American Mosquito Control Association*, **9**, 196–205.
- Christophers, S.R. (1960) *Aedes aegypti* (L.), the yellow fever mosquito; its life history, bionomics and structure. Cambridge University Press, London.
- Costero, A., Edman, F.D., Clark, G.G. & Scott, T.W. (1998) Life table study of *Aedes aegypti* (Diptera: Culicidae) in Puerto Rico fed only human blood versus blood with sugar. *Journal of Medical Entomology*, **35**, 809–813.
- Daugherty, M.P., Alto, B.W. & Juliano, S.A. (2000) Invertebrate carcasses as a resource for competing *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, **37**, 364–372.
- Day, J.F., Edman, J.D. & Scott, T.W. (1994) Reproductive fitness and survivorship of *Aedes aegypti* (Diptera: Culicidae) maintained on blood with field observations from Thailand. *Journal of Medical Entomology*, **31**, 611–617.
- Edman, J.D., Strickman, D., Kittayapong, P. & Scott, T.W. (1992) Female *Aedes aegypti* (Diptera: Culicidae) in Thailand rarely feeds on sugar. *Journal of Medical Entomology*, **29**, 1035–1038.
- Foster, W.A. (1995) Mosquito sugar feeding and reproductive energetics. *Annual Review of Entomology*, **40**, 443–474.
- Foster, W.A. & Eischen, F.A. (1987) Frequency of blood feeding in relation to sugar availability in *Aedes aegypti* and *Anopheles quadrimaculatus* (Diptera: Culicidae). *Annals of the Entomological Society of America*, **80**, 103–108.
- Gary, R.E. & Foster, W.A. (2001) Effects of available sugar on the reproductive fitness and vectorial capacity of the malaria vector *Anopheles gambiae* (Diptera: Culicidae). *Journal of Medical Entomology*, **38**, 22–28.
- Gary, R.E. & Foster, W.A. (2004) *Anopheles gambiae* feeding and survival on honey dew and extra-floral nectar of peridomestic plants. *Medical and Veterinary Entomology*, **18**, 102–107.
- Gomes, A.C., Silva, N.N., Marques, G.R.A.M. & Brito, M. (2003) Host-feeding patterns of potential human disease vectors in the Paraíba Valley Region, State of São Paulo, Brazil. *Journal of Vector Ecology*, **28**, 74–78.
- Harrington, L.C., Buonaccorsi, J.P., Edman, J.D., Costero, A., Kittayapong, P., Clark, G.G. & Scott, T.W. (2001a) Analysis of survival of young and old *Aedes aegypti* (Diptera; Culicidae) from Puerto Rico and Thailand. *Journal of Medical Entomology*, **38**, 537–547.
- Harrington, L.C., Edman, J.D. & Scott, T.W. (2001b) Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? *Journal of Medical Entomology*, **38**, 411–422.
- Hawley, W.A. (1988) The biology of *Aedes albopictus*. *Journal of the American Mosquito Control Association*, **4** (Suppl.), 1–40.
- Juliano, S.A. (1998) Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? *Ecology*, **79**, 255–268.
- Juliano, S.A., O'Meara, G.F., Morrill, J.R. & Cutwa, M.M. (2002) Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. *Oecologia*, **130**, 458–469.
- Juliano, S.A., Lounibos, L.P. & O'Meara, G.F. (2004) A field test for competitive effects of *Aedes albopictus* and *Aedes aegypti*. South Florida: Differences between sites of coexistence and exclusion? *Oecologia*, **139**, 583–593.
- Klowden, M.J. (1987) Effect of sugar deprivation on the host seeking behaviour of gravid *Aedes aegypti* mosquitoes. *Journal of Insect Physiology*, **32**, 479–483.
- Klowden, M.J. & Chambers, G.M. (1992) Reproductive and metabolic differences between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, **29**, 467–471.
- Lounibos, L.P., Suárez, S., Menéndez, Z., Nishimura, N., Escher, R.L., O'Connell, S.M. & Rey, J.R. (2002) Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*? *Journal of Vector Ecology*, **27**, 86–95.
- Martinez-Ibarra, J.A., Rodriguez, M.H., Arredondo-Jimenez, J.I., Marina-Fernandez, C.F. & Yuval, B. (1996) Importance of nectar in the dispersal of *Aedes aegypti*. *Journal of the American Mosquito Control Association*, **12**, 465–466.
- Niebylski, M.L., Savage, H.M., Nasci, R.S. & Craig, G.B. (1994) Blood hosts of *Aedes albopictus* in the United States. *Journal of American Mosquito Control Association*, **10**, 447–450.
- Packer, M.J. & Corbet, P.S. (1989) Size variation and reproductive success of female *Aedes punctator* (Diptera: Culicidae). *Ecological Entomology*, **14**, 297–309.
- SAS Institute Inc. (1989) *SAS/STAT User's Guide*, Version 6 4th edn, Vol. 2. SAS Institute, Cary, NC.
- Scott, T.W., Clark, G.G., Amerasinghe, P.H., Lorenz, L.H., Reiter, P. & Edman, J.D. (1993a) Detection of multiple blood feeding patterns in *Aedes aegypti* (Diptera: Culicidae) during a single gonotrophic cycle using histological techniques. *Journal of Medical Entomology*, **30**, 94–99.
- Scott, T.W., Chow, E., Strickman, D., Kittayapong, P., Wirtz, R.A., Lorenz, L.H. & Edman, J.D. (1993b) Blood feeding patterns of *Aedes aegypti* (Diptera: Culicidae) in a rural Thai village. *Journal of Medical Entomology*, **30**, 922–927.
- Scott, T.W., Naksathit, A., Day, J.F., Kittayapong, P. & Edman, J.D. (1997) A fitness advantage for *Aedes aegypti* and the viruses it transmits when females feed only on human blood. *American Journal of Tropical Medicine and Hygiene*, **57**, 235–239.
- Straif, S.C. & Beier, J.C. (1996) Effects of sugar availability on the blood-feeding behavior of *Anopheles gambiae* (Diptera: Culicidae). *Journal of Medical Entomology*, **33**, 608–612.
- Tempelis, C.H. (1975) Host-feeding patterns of mosquitoes, with a review in analyses of blood meals by serology. *Journal of Medical Entomology*, **6**, 635–653.
- Van Handel, E., Edman, J.D., Day, J.F., Scott, T.W., Clark, G.C., Reiter, P. & Lynn, H.N. (1994) Plant sugar, glycogen, and lipid

assay of *Aedes aegypti* collected in urban Puerto Rico and rural Florida. *Journal of the American Mosquito Control Association*, **10**, 149–153.

Yee, W.L. & Foster, W.A. (1992) Diel sugar-feeding and host-seeking rhythms in mosquitoes (Diptera: Culicidae) under

laboratory conditions. *Journal of Medical Entomology*, **29**, 784–791.

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