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Host species diversity and post-blood feeding carbohydrate availability enhance survival of females and fecundity in *Aedes albopictus* (Diptera: Culicidae)

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Abstract

Aedes albopictus mosquito is an opportunistic blood feeder and has a broad host range. The feeding behavior and habits of this mosquito are liable to increase the transmission potential of arboviruses. The survival and fecundity in *A. albopictus* fed on different hosts and post-blood meal provision of sugar were investigated in a laboratory-reared colony. Adult survival of caged female *A. albopictus* that were fed on blood of two different hosts (double meal) was higher than the females fed only on one host (single meal) (mean survival: 70.2 ± 9.6 vs. 55.5 ± 5.5%, respectively) when held in the laboratory for 72 h after blood feeding. Mean survival of females provided 10% sucrose solution (in water) after a single or double blood meal was higher (90.5 ± 6.4% and 89.3 ± 6.5%, respectively) than in the respective groups receiving water only following blood feeding (double meal: 49.0 ± 9.6%; single meal: 45.3 ± 10.9%). Females receiving a double meal were more fecund on average (89.0 ± 6.6 eggs) than females provided a single meal (82.3 ± 8.2 eggs).
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Index Descriptors and Abbreviations: Mosquitoes; *Aedes albopictus*; Diverse blood meals; Reproduction; Adult female longevity

1. Introduction

Aedes albopictus Skuse primarily poses a biting nuisance in the USA. However, north American strains of *A. albopictus* have experimentally shown a high degree of vector competence to Dengue fever virus, Rift Valley fever virus, eastern equine encephalitis virus, yellow fever virus, and some other arboviruses (Mitchell, 1991). This mosquito feeds opportunistically and has a broad host range; it has been documented that >92% of the field-collected *A. albopictus* females fed on blood of mammals (Niebylski et al., 1994; Savage et al., 1993). Also, human host attack rate and blood feeding activity of *A. albopictus* are influenced by the mosquito age, body size, time of the day, blood engorgement state, and carbohydrate availability (Xue et al., 1995; Xue and Barnard, 1996, 1999).

Blood and sugar are essential for survival and reproduction in female mosquitoes (Briegel and Horler, 1993; Foster, 1995). Blood feeding to repletion on different host species (double meal) is a frequent occurrence in mosquitoes (Edman and Downe, 1964; Xue and Edman, 1991). Because a double blood meal increases the number of host contact events by a single female (Klowden, 1988; Xue and Edman, 1991), such behavior in the mosquito population can affect vectorial capacity (Boreham and Garrett-Jones, 1973; Scott et al., 1993). Some of the factors associated with blood feeding to repletion on different host species have been characterized (e.g., Scott et al., 1993; Klowden and Briegel, 1994; Xue et al., 1995) but relatively little is known concerning the survivorship and fecundity of such

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mosquitoes when they have access to carbohydrate subsequent to blood meal. In the present study, we tested the hypothesis that sugar availability to female *A. albopictus* following single (one host species) or double (two host species) blood meals did not influence survival of the females or their fecundity. Knowledge of any such influence(s) will improve our understanding of how blood meals from diverse host species affect survivorship and fecundity in the mosquito population. It will also allow more precise assessments of the risk of transmission of mosquito-borne disease agents to humans and animals.

2. Materials and methods

2.1. Mosquitoes

The *A. albopictus* used for this study were from a laboratory colony maintained [27 ± 2 °C, 70% relative humidity, and 14:10 h (L:D) photoperiod] since 1992 at the insectary of the USDA-ARS-Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida, USA. Larvae were reared in batches of 200 per plastic tray (30 × 19 × 5 cm high and containing 1000 ml well water) as described in Gerberg et al. (1994). Adults emerged directly into screened stock cages and were provided continuous access to 10% sucrose/water solution. For colony maintenance, females were weekly blood fed on restrained 5- to 7-wk-old chicks.

2.2. Experimental unit

In all studies, the experimental unit comprised a clear plastic cup (5 cm diam. × 4 cm long; volume 78.4 cm^3) with 1.7-mm mesh cloth covering the open end. Each cup contained a single female. A typical observation comprised placement of the experimental unit (mesh cloth end) directly against the skin of a restrained (in supine position) chicken and/or guinea pig until achieving the desired objective of an experiment.

2.3. Effects of blood-meal type (single or double) on adult survival

The purpose of this experiment was to determine adult survival in test populations of mosquitoes after complete blood engorgement on a chicken (single meal), and on partial blood engorgement on a chicken with completion of the blood engorgement on a guinea pig (double meal). To accomplish this, two groups of 25 cups (one female per cup) were used. Each female in a cup in one group was exposed to a restrained chicken until full engorgement was achieved as evidenced by the female's complete withdrawal of mouthparts from the host skin. Females in the second group were first exposed to a restrained chicken for a partial engorgement, and then allowed to complete engorgement on a guinea pig. The criterion for partial engorgement during blood feeding was the visible gradual expansion of the blood meal to the 4th abdominal segment (Xue et al., 1995). At this indication, blood feeding was interrupted and the female allowed to feed to repletion on a guinea pig. After blood feeding, all females were kept in the insectary for 72 h at which time the number of surviving mosquitoes was checked and recorded. The experiment was repeated nine times.

2.4. Effects of blood-meal type (single or double) and provision of water and sugar–water after the meals on adult survival

The objective of this experiment was to determine adult survival in test populations of mosquitoes provided with only water and sugar–water (10% sugar solution), after single (chicken) or double (chicken and guinea pig) blood meals. For this test, a total of 100 cups with each cup containing one female was prepared. The cups were divided into four groups of 25 cups each and each group placed in a plastic tray (56 × 43 × 8 cm), thus utilizing four trays. All four trays were lined with paper towels. Towels in two trays were soaked to run off with a 10% sucrose solution, while in the other two trays, the towels were soaked to run off with water only (Xue and Barnard, 1999). Females in two groups of 25 each were blood fed on one host (chicken) as above and cups of one group were placed in the tray with 10% sugar solution, and the other group in the tray with water only. Similarly, females in the other two groups of 25 each were blood fed on two hosts (chicken and guinea pig, as above) and one group of cups was placed in the tray with 10% sugar solution, and the other group in the tray with water only. At 72 h after the blood meal, adult survival in each cup was checked and recorded.

2.5. Effects of blood-meal type (single or double) on fecundity

The experimental design for the fecundity study was identical to that described above except that at 72 h post-blood-feeding, the living females were killed by freezing and a randomly selected sample of 10–15 females was dissected to count and record the number of stage five ovarioles. The fecundity experiment was repeated six times.

2.6. Statistical analysis

A matched-pairs *t*-test (PROC MEANS: SAS, 2003) was used to determine the effect of single or double blood meal on the survival of female mosquitoes deprived of water or sugar–water (experiment 1), and on the effect of single or double blood meal on the fecundity of female mosquitoes provided sugar–water (experiment 3). In experiment 2, we determined the effect of single or double blood meal and the provision of water, or sugar–water on female survival as a 2 × 2 factorial experiment. In this case, the set of all treatment combinations (water only + single blood

meal; water only + double blood meal; sugar–water + single blood meal; sugar–water + double blood meal) was applied to the experimental units using a completely randomized design and the responses analyzed using ANOVA (PROC GLM: SAS, 2003).

3. Results

In experiment 1, the effect of single or double blood meal on the survival of female mosquitoes was significant (mean difference = 14.7 ± 3.5%, $t = 4.23$, Prob > $t = 0.0029$) (Table 1). This means that among all of the females allowed either to (1) complete feeding on a chicken (single meal), or (2) interrupted from feeding on a chicken and then allowed to complete feeding on a guinea pig (double meal), more of the females in the latter group (70.2 ± 9.6%) were alive after 72 h, compared with the females in the former group (55.5 ± 5.5%). From this result, we infer that a double meal increases survival in female *A. albopictus*.

The fitted model for the effect of blood meal number (single or double) and the provision of water, or sugar–water on female survival (experiment 2) was significant ($F_{3,23} = 49.72$, $P < 0.001$) (Table 2). These results indicate that the provision of water vs. sugar–water affected survival in females ($F_{1,23} = 148.57$, $P < 0.001$) regardless of whether the females acquired a single or double blood meal. The mean survival rate for females that received water only was 47.2%. This was significantly different (LSD test, $P = 0.05$) from females receiving the sugar–water solution, whose mean survival rate was 89.9%. Percent survival was not significantly influenced ($F_{1,23} = 0.13$, $P = 0.725$) by the number of blood meals the female acquired, regardless of whether the female received water only or sugar–water solution. Mean percent survival in females receiving a single blood meal (chicken) was 67.9%, which was not significantly different (LSD test, $P = 0.05$) from the mean survival (69.2%) for females receiving blood from chicken and then guinea pig. There was no significant interaction between the effect of water, sugar–water and the effect of blood meal number ($F_{1,23} = 0.47$, $P = 0.498$), which means that survival responses to the number of blood meals was relatively consistent at each treatment level of water, sugar–water, and vice-versa.

In experiment 3, the effect of single or double blood meal on the fecundity of female mosquitoes that were provided sugar–water was significant (mean difference = 6.7 ± 3.6 eggs, $t = 4.23$, Prob > $t = 0.0082$) (Table 3). This means that among all of the females that were allowed either to feed to completion on a chicken (single blood meal) or interrupted from feeding on a chicken and then allowed to complete feeding on a guinea pig (double blood meal), the females in the latter group matured significantly more eggs than the females in the former group (89.0 ± 6.6 vs. 82.3 ± 8.2 eggs/female). From this result, we can say that females receiving a double blood meal mature more eggs than those receiving a single blood meal.

Table 1
Effect of single (one host) and double (two hosts) blood meal on survival of caged female *Aedes albopictus* at 72 h after the blood meals in the laboratory

Blood meal type	Number females tested	Survival (%) mean[a] ± SD
Single	300	55.5 ± 5.5a
Double	300	70.2 ± 9.6b

[a] Means followed by the same letter are not significantly different (*t*-test, $P = 0.05$).

Table 2
Effect of single (one host) and double (two hosts) blood meal and provision of water or sugar–water on survival of caged female *Aedes albopictus* at 72 h after the blood meals in the laboratory

Blood meal type	Number females tested	Survival (%) mean ± SD
Water		
Single	150	45.3 ± 10.9
Double	149	49.0 ± 9.6
Sugar–water		
Single	149	90.5 ± 6.4
Double	149	89.3 ± 6.5

Sources of variation for the ANOVA: Water only versus water plus sugar ($F_{1,23} = 148.57$, $P = <0.001$), number of blood meals ($F_{1,23} = 0.13$, $P = 0.725$), interaction ($F_{1,23} = 0.47$, $P = 0.498$).

Table 3
Effect of single (one host) and double (two hosts) blood meal on fecundity of caged female *Aedes albopictus* at 72 h after the blood meals in the laboratory

Blood meal type	Number females tested	Mean[a] number of eggs ± SD
Single	72	82.3 ± 8.2a
Double	70	89.0 ± 6.6b

[a] Means followed by the same letter are not significantly different (*t*-test, $P = 0.05$).

4. Discussion

In the USA, mosquito host-feeding studies conducted by using ELISA and precipitin test methods in Missouri, Florida, Indiana, Illinois, and Louisiana discovered that wild *A. albopictus* fed opportunistically on blood of a variety of hosts (Niebylski et al., 1994). Partial blood engorgement and post-blood meal sugar availability affect the continued blood feeding behavior of this mosquito (Xue and Barnard, 1999). These criteria (host-seeking and blood-feeding behavior and habits) are conducive to increasing the potential for transmission of pathogens by this mosquito.

Survivorship in vector mosquitoes is related to temperature and available nutrition (Clements, 1992). It had been documented in laboratory populations of *Aedes aegypti* L. that a single blood meal could maintain adult survivorship for a relatively long time (Nayar and Sauerman, 1975; Day et al., 1994); however, the influence of double meal in this regard is not known. Our results show that female *A. albopictus* obtaining a double meal live longer than those obtaining a single meal and produce significantly

more eggs. Higher survivorship and fecundity in the former case may be related to blood concentration enhancement between the two feedings and possible improvement of overall nutritional value.

Not surprisingly, availability of sugar after both single and double meals increased female survivorship compared with the corresponding groups of females provided water only following blood engorgement. This difference in feeding behavior from *A. aegypti* L., which in Thailand rarely feeds on sugar (Edman et al., 1992), may explain why the latter species rests mainly indoors following a blood meal, whereas *A. albopictus* rests outdoors in vegetation after blood feeding, perhaps to obtain sugar (Lu, 1990).

Any changes in mosquito reproductive potential could influence vector population ecology. The effects of host species diversity (i.e., single, double, or > double blood meal) on mosquito reproduction are complicated because of the many other factors that influence fecundity. The fecundity increase in *A. albopictus* obtaining a double meal may be caused by the prediuresis expulsion of fluid (and resultant blood meal concentration) and may provide a mechanism to compensate for host anemia. Another factor may be that the different blood source(s) may have different nutritional values. Also, interrupted feeding behavior may enable sufficient protein intakes to occur to overcome the threshold requirement for initiation of a gonotrophic cycle.

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