ABSTRACT: We determined the blood meal size - fecundity relationship in *Anopheles gambiae* in the laboratory. Our primary interest was to determine whether the fecundity curve has a non-linear component, i.e., does it decelerate towards an asymptote? Small and large adult females in their second gonotrophic cycle were fed on human hosts for predetermined lengths of time. Blood meal size was ascertained by weight and subsequent fecundity was determined by the number of eggs laid on filter paper. In both small and large-bodied females there was a significant curvilinear component. We used this relationship to show that marginal returns from feeding decline as a function of current blood load. This means that further fecundity payoff for continued feeding declines over time. *Journal of Vector Ecology* 30 (1) : 83-86. 2005.

**Keyword Index:** *Anopheles*, blood meal, fecundity curve, body size.

INTRODUCTION

All living organisms face tradeoffs (Roff 1992). These tradeoffs vary dramatically from reproduction versus survivorship (Roitberg 1989) to size versus numbers of offspring (Smith and Fretwell 1974), to name just two such relationships. Most importantly, tradeoffs generally force organisms to compromise. Because there is no such thing as a free lunch, many animals compromise their feeding behavior by altering where, how, or when they feed. Determining how and why such compromises evolve is the focus of behavioral ecology (Krebs and Davies 1987).

Mosquitoes face tradeoffs when blood feeding. On the one hand, gametic (fecundity) and somatic (e.g. energy reserves) payoffs increase with the size of the blood meal (Briegel 1990a, 1990b, Hurd et al. 1995, Naksathit et al. 1999). On the other hand, due to increased mass from feeding, susceptibility to predation also increases as a function of blood meal size (Roitberg et al. 2003). Thus, Roitberg et al. (2003) suggested that female mosquitoes might imbibe less than maximum-sized blood meals under risk of predation (also see Anderson and Roitberg 1999).

From the payoff perspective, there are two issues associated with blood feeding: one is the sign (positive in this case) and the other is the curvature of the payoff function. Curvature is important with regard to the response to tradeoffs. If for instance, fecundity increases linearly with blood meal size, then the marginal returns from imbibing more blood are independent of current blood state. In that case, we would expect mosquitoes to express a single fixed response to a given degree of danger while feeding because the gain from further feeding would be the same, for example, whether an individual’s midgut is one-quarter or one-half full. On the other hand, if the payoff curve decelerates, then the marginal returns from imbibing more blood will decline with current midgut state. There will be less to gain from further feeding at high versus low blood meal state. Here we would predict that mosquitoes should be more willing to abandon hosts if disturbed when mostly, versus partially, full. This is a non-intuitive prediction because it becomes more difficult to fly from hosts as mass increases.

There is good evidence that the fecundity payoff curve from blood feeding is positive for anopheline mosquitoes in particular and female mosquitoes in general (Briegel 1990a). What is less clear is whether the payoff function decelerates up to a maximum. Briegel (1990a) and Takken et al. (1998) provide some excellent data on the subject, but since neither of these experiments were designed to test for curvature they cannot be applied unequivocally as the individuals were not randomly assigned a feeding regime, but rather they fed *ad lib* and chose when to terminate feeding, and only four blood meal categories were considered, respectively. Additionally, because of different energetic challenges, we might expect to see differences in the payoff curves for small versus large-body individuals both in the slopes and asymptotes, with small individuals reaching the asymptote sooner and at a lower level than their large-body counterparts.

The purpose of this paper is to determine if, in fact, the blood meal fecundity relationship decelerates as a curvilinear function. We determined this by experimentally manipulating body size and blood meal size under laboratory conditions. We found that the blood meal size-fecundity relationship is curvilinear but only relative to body size.

MATERIALS AND METHODS

We used the mosquito *Anopheles gambiae* Ifakara strain, which has been in laboratory culture for about four years. Larvae were reared in pans of distilled water and provided with fish food (Tetramin™) daily. After approximately five days, larvae developing into pupae were transferred to 27000 cm² screen and Plexiglas™ cages. Adults of both sexes were fed 10% sugar water through braided cotton rolls for sustenance. Mating occurred within two d of eclosion within
the maintenance cages. Once per wk, the investigators allowed female mosquitoes to blood feed on their arms for 20 min. Following blood feeding, we provided mosquitoes with moist filter paper (9 cm/D) as an oviposition substrate. Three days later, eggs were collected from filter paper, allowed to incubate in glass bowls for two to three days, and transferred to rearing pans. The mosquito larvae and adults were kept in a Conviron™ walk-in environmental chamber at 28 ± 2 °C and 60 ± 5 % RH and 12:12 L:D photoperiod.

We produced two body class sizes using the methods of Lyimo and Takken (1993). Large-body individuals were produced by rearing larvae at low densities with large amounts of food, whereas small-body individuals were produced under high larval density and low food conditions. Eggs were removed from a maintenance cage, allowed to incubate, and placed in a rearing pan (42 cm x 28 cm x 5 cm) full of distilled water. At the first instar stage, approximately 100 larvae were removed and placed in an identical pan, thus simulating rearing environments with high and low densities. Low-density larvae were provided with 0.3 mg food/larvae/d and high-density larvae were provided with 0.1 mg food/larvae/d. The food was weighed with a standard laboratory balance (Sartorius GMBH Gottingen). Pans were monitored for larval mortality and food amounts were adjusted accordingly. Water was replaced when necessary to maintain constant water levels. Pans were cleaned periodically via siphoning to remove decaying food. This rearing method produced large-body individuals whose wing length was significantly greater than those in the small-body class (3.44 mm ± 0.02 S.E. vs. 2.99 mm ± 0.02 S.E., t = 13.6, p<0.001). After pupation, individuals from both trays were removed with an eyedropper and placed into water-filled glass bowls, which were placed in maintenance cages specified for low and high-density individuals.

Three to five d after eclosion, females were offered blood meals on a human host. Individuals that fed were then provided with oviposition sites and maintained with sugar water for five to seven more days. At this point, we replaced the sugar water with distilled water for 24 h to increase the likelihood that each individual would feed on the host blood. Following this deprivation period, we removed females from the rearing cages and placed them individually in 20 ml glass scintillation vials. Each individual was chilled for ca. 1.5 min at 5 °C and weighed to the nearest 0.001 mg on a Cahn balance. Individuals were then allowed to warm to room temperature for at least five minutes. Following this warm-up period, individuals were taken to the laboratory and fed on a human arm or foot. We randomly predetermined both the feeding site and length-of-feed by lottery draw of paper chits.

Feeding was facilitated by placing the inverted glass holding tube at the feeding site. Most mosquitoes attempted to feed soon after placement. If they failed to feed within two min we put them aside and chose another individual. Within 30 min of failure to Initially feed, we provided second opportunities. If the individual failed to feed, we gave it one more chance and if still not successful, it was rejected. Following the predetermined length of feed, we chilled each individual again and reweighed it on the Cahn balance. For those that fed, we recorded the length of feed as well as the length of time in prediuresis. We did not attempt to measure the amount of liquid excreted during this phase for fear of disturbing the animals. Following completion of feed we placed each individual in her own 14 x 14 x 14 cm Plexiglas-screen cage with sugar water and an oviposition site as described above and returned them all to the environmental chamber. Several days later, eggs were counted and recorded.

We tested for curvature by applying a quadratic curve fitting procedure with blood meal mass, as well as proportional mass (blood meal mass/ wet body mass), using the Fit Y by X procedure in JMPIN 4.0.4 (SAS Institute). We did this in two ways. First, we forced the regressions through the origin because fecundity must equal zero when blood meal size is zero. Unconstrained regressions gave positive intercepts (i.e., non-zero fecundity with blood meal size equal zero), a biological impossibility. In addition, we removed all zero fecundity values except when we fed a blood meal of size zero. Our reasoning here was that zero fecundity may be a qualitative or a quantitative response and we have no way of distinguishing between them. In other words, zero fecundity can be caused by insufficient blood to produce a meal or by some other non-blood factor (e.g., non-mated status). Employing an alternative interpretation, we considered Briegel’s (1990b) suggestion that a minimum blood meal size is necessary for egg maturation. Support for this notion came from a highly significant logistic regression for an oviposition event as a function of blood meal size ($\chi^2 = 24.4$, df = 1, p<0.001). Following visual inspection of the data, we set the intercept perpendicular to the smallest positive blood meal–fecundity point and fit curves through this intercept. Note that in neither case is the exact derivation of the fecundity curve our goal but rather whether the second order (quadratic) term is statistically significant.

In addition to the above, we applied a stepwise multiple regression with fecundity as the response variable and blood meal mass, proportional blood meal mass, wing length, mosquito dry weight, and duration of prediuresis as independent variables (JMPIN 4.0.4 - SAS Institute). We indexed the blood meal by absolute and relative mass for convenience and perspective. With regard to the latter, we were interested in how and why mosquitoes make host desertion decisions. Thus, we were looking for simple rules and proxies of fitness (see Roitberg et al. 2001) that female mosquitoes might utilize in nature. While it is possible that anopheline females track hemat (Briegel 1990a), we sought plausible though admittedly less exact rules that could facilitate such decisions. Similarly, we investigated the blood feeding payoff function during the second gonotrophic cycle to remove any size-dependent effects of pre-gravid feeding that are so common in anophelines (Takken et al. 1998). Again, while this effect is interesting in its own right, it is outside of our realm of interest.

RESULTS

Virtually all (72/73) large-body females that blood fed, oviposited, whereas only 66 of 98 small-body females laid
eggs ($\chi^2 = 26.3$, $p<0.001$). A logistic regression for small body females did not show an effect of body size on likelihood of oviposition ($\chi^2 = 0.28$, df = 1, $p=0.5$). As noted earlier, there was a significant inverse effect of absolute and proportion blood meal size on likelihood of oviposition.

When the regression was constrained through the origin, there was a significant quadratic term in the regressions for fecundity as a function of proportion body mass for both large ($p < 0.001$) and small-body ($p<0.02$) individuals (fecundity $= 0 + 77.67 \times 3 - 18.81 x^2$) and (fecundity $= 0 + 57.43 y - 12.50 y^2$, respectively). Because the regression is constrained, it was not possible to determine $r^2$ values. In neither case was absolute blood meal size a significant quadratic term ($p<0.1$).

When the regression was forced to the right of the origin, there was a significant quadratic term in the regressions for fecundity as a function of blood meal mass for both large ($p < 0.001$) and small-body ($p<0.02$) individuals (fecundity $= 0.35 + 84.7 x - 14.9 x^2$ and (fecundity $= -30 + 94.6 - 22.3 x^2$, respectively) (Figures 1a, 1b). Proportion of total mass contributed by blood meal mass also provided significant quadratic terms for small and large individuals ($p<0.0001$).

Stepwise multiple regressions on small-body female fecundity included only blood meals in the model ($p<0.0005$). By contrast, stepwise multiple regression on large-body female fecundity included the following variables: prediuresis time ($0.0042$), wing length ($0.023$), and absolute blood meal size ($0.0533$).

**DISCUSSION**

Our experiment demonstrates that *Anopheles gambiae* mosquitoes receive decelerating fecundity returns from imbibing increasingly large amounts of blood. This is true for large and small-body individuals. The implication from this demonstration is that feeding persistence decisions are expected to be state dependent, i.e., response to disturbance should depend upon the amount of blood in the midgut. We are currently evaluating this hypothesis and will report on it elsewhere.

Figure 2 shows the marginal returns that large and small *Anopheles gambiae* females receive from feeding as a proportion of body mass. This graph was derived from the quadratic regressions of fecundity against blood meal mass. Several features should be noted. First, as postulated, after the initial gains from initiating oviposition, the functions are negative, i.e., the more full a mosquito’s midgut, the less extra fecundity it gains from further blood feeding. Second, as expected, the curve is shifted to the right for small-body individuals. This indicates that larger blood meals are required for small individuals to initiate oviposition. Third, the marginal returns curve is steeper for small-body individuals. As a result, those individuals arrive at their asymptote sooner as they imbibe blood. Taken together, we predict that both small and large mosquitoes should give up and leave hosts if disturbed (Walker and Edman 1985) but that this tendency should increase as a function of current blood meal state particularly for small individuals.

Absolute blood meal size and proportional weight change were good predictors of fecundity, but only the latter had a significant second order (i.e., non-linear) term in the regression constrained at origin. It is not clear why this is so, but in another study on this same colony proportional weight change was the best predictor of flight response to disturbance (Roitberg et al. 2003).

Our use of blood mass as the independent variable in the fecundity function can be criticized because it ignores prediuresis and thus underestimates blood meal size. We offer several defenses. First, as noted earlier, we employed a protocol that aimed to minimize disturbance. This is particularly important because anophelines are more easily disturbed the heavier they become (Macdougal and Roitberg, pers. obs.) making it very difficult to obtain the very large blood meals that were hypothesized to generate the asymptotic part of the fecundity curve. Second, our goal was not to derive the quantitatively precise fecundity curve but rather its qualitative shape. Finally, omission of the excretion behavior will bias blood concentration toward larger blood meals because this behavior is rare when blood meals are small. In essence, our experiment is a very conservative test of the curvature hypothesis because more concentrated meals are nutritionally superior (Briegel 1985).
Our results agree with those of Takken et al. (1998). We also found that small individuals were far less likely to mature eggs and when they did, fecundity was less (40.3 ± 3.7 versus 55.7 ± 3.8, t = -2.1, p < 0.05). However, on an efficiency basis, small-body individuals converted absolute units of blood into eggs as effectively as their large-body counterparts (34.4 ± 2.7 versus 34.0 ± 2.4 eggs/μl blood, t = -0.07, n.s.). This result is in contrast to that of Takken et al. (1998) who showed that larger individuals had higher conversion efficiencies. Of course, our work differs from theirs in that we worked within the second gonotrophic cycle, whereas the former study focused on the first cycle.

A decelerating fecundity payoff curve suggests some limiting resources cause an upper limit to fecundity regardless of blood volume intake. What might this factor(s) be? Briegel (1985) suggested that isoleucine is in short supply in human blood and, as a result considerable blood protein is diverted to extraovarian processes. Whether limited isoleucine shortages can cause increased diversion as a function of blood volume remains to be seen.

Finally, we might consider our findings in the context of disease transmission. Decelerating returns and likelihood of host response to feeding will determine the size and frequency of blood meals. These parameters are key to the epidemiology of mosquito-vectored diseases (Anderson and May 1992).

Acknowledgments

Eva Poon and Christy Macdougal helped with mosquito colony maintenance. This work was supported by a grant from NSERC, Canada. We thank Carl Lowenberger, Elizabeth Elle, and anonymous reviewers for comments on an earlier version of this paper.

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