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Pouncing spider, flying mosquito: blood acquisition increases predation risk in mosquitoes

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Female mosquitoes dramatically increase their mass when blood feeding on their hosts. Such an increase could impact mosquito mortality risk by reducing escape speed and/or agility. We used two laboratory-based experiments to test this notion. In the first, we allowed mature female *Anopheles gambiae* mosquitoes to feed from 0 to 4 min and then attacked those females with an artificial predator. We videotaped subsequent escape response of each mosquito. Analysis of those responses clearly demonstrated an inverse relationship between increased mass and escape speed. In the second experiment, we exposed both blood-engorged and unfed *A.gambiae* females to single zebra spiders (*Salticus scenicus*) in small plexiglass cages. Here, we focused on mosquito escapes from searching and pouncing spiders. We found that engorged mosquitoes were three times less likely to escape searching spiders compared to unfed conspecifics. Thus we conclude that blood feeding has substantial state-dependent risk both at the host (experiment 1) and after feeding (experiment 2). Such risk can be extended to a broad range of taxa. *Key words: Anopheles*, escape, feeding, flight, mass, mosquitoes, predation risk, spiders. *[Behav Ecol 14:736–740 (2003)]*

H eterotrophic organisms must acquire nutrients to maintain somatic and gametic function. Nutrient acquisition, however, has multiple effects on an organism: it alters the host's energy or nutrient state (Mangel and Clark, 1988) and also causes physical changes in the organism. For example, honeybee mass can increase by 40% during a single foraging bout (Winston, 1987). Similarly, the weight of birds can vary by as much as 10% over a single day (e.g., zebra finch; Metcalfe and Ure, 1995). At the extreme, migratory mass of European robins (*Erithacus rubecula*) can exceed that of similar-age nonmigratory individuals by as much as 50% (Lind et al., 1999). Are there costs to such weight gains? Does increased mass impair flight and thus increase an organism's risk of predation?

A consensus is emerging that daily weight gain is modest and does not increase predation risk in birds (Veasey et al., 1998). In contrast, the more substantial migration-related weight changes can dramatically increase risk to predation (Lind et al., 1999). For migratory birds, however, it is difficult to ascribe changes in flight (escape) ability to weight change per se because of concomitant changes in muscle mass, digestive organs, and so on (Guglielmo and Williams, 2003; McWilliams and Karasov, 2001). Thus, to study the effect of mass gain on predation risk directly, it may be useful to study other taxa where change is rapid and large. Here we evaluated whether substantial short-term weight gains increase predation risk for the blood-feeding mosquito, *Anopheles gambiae*.

Most adult female mosquitoes blood feed to obtain the required protein for maturing eggs. During a single feeding bout, lasting 4 to 5 min, a female anopheline mosquito can increase mass by more than 200% (see below). This increase is not due solely to the volume of blood ingested, as anophelines can also excrete the water component of the plasma (prediuresis) to concentrate the number of hematocytes in their midgut. If blood-engorged mosquitoes experience impaired flight ability due to this sudden weight gain, they may be at risk from two different sources. First, a slow-moving mosquito may be very susceptible to attack from a host, either for defensive reasons (i.e., pecking, tail swishing, swatting, etc.; Edman et al., 1984) or if hosts prey upon those very mosquitoes (e.g., birds and rodents; Guinan and Sealy, 1986). Second, should a mosquito successfully feed upon a host, the extra mass must be carried for several hours while the blood meal is processed. During that period a mosquito may be disproportionately susceptible to general predators if escape abilities are compromised by increased mass.

In this article, we describe two laboratory experiments designed to test the notion of increased predation risk to blood-fed mosquitoes both at the host and away from the host (i.e., from humans and jumping spiders, respectively). Despite the large anecdotal literature on impeded flight in engorged mosquitoes (see Spielman and D'Antonio, 2001) this is the first manipulative experiment to directly test this idea.

METHODS

For experiments, we used the mosquito *Anopheles gambiae* Ifakara, which has been in laboratory culture for about 3 years. Larvae were reared in pans of distilled water and provided with fish food (Tetramin™) daily. After approximately 5 days, larvae developing into pupae were transferred to 27,000-cm^3 screen and plexiglass cages. Adults of both sexes were fed 10% sugar water, through braided cotton rolls for sustenance. Mating occurred within 2 days of eclosion within the maintenance cages. Once per week, B.D.R. allowed female mosquitoes to blood feed on his arm for 20 min. Following blood feeding we provided mosquitoes with moist filter paper (9 cm/diam) as an oviposition substrate. Three days later, we collected eggs from filter paper 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% relative humidity, and 12:12 h light:dark photoperiod.

We used the zebra spider (*Salticus scenisus*) as our generalist predator. These spiders were collected from walls near vegetation on the Simon Fraser University (SFU) campus in Burnaby, British Columbia, Canada. Individuals were placed in 8.5 cm i.d. × 1 cm high Petri dishes and supplied with braided cotton roll moistened with distilled water. We collected every 3 days prey from vegetation at the SFU campus. Each spider was supplied with aphids, flies, plant hoppers, and parasitoid wasps. Spiders were never fed mosquitoes. We maintained spiders in these Petri dishes for approximately 10 days at 19–23°C, 30–70% relative humidity, and 16:8 h light:dark until experiments began.

All experiments were conducted in the laboratory at 25–28°C, 30–70% relative humidity. Central heating and small portable heaters placed within 0.5 m of the experimental arenas regulated temperature. For both experiments, trials were run from 1300 to 1600 h under fluorescent lights, which coincided with onset of evening in the walk-in chamber.

Experiment 1: does mosquito mass change alter chance of escape from the host?

Adult female mosquitoes reaching 3–5 days of age were blood-fed by B.D.R. Two days later, these mosquitoes were allowed to oviposit. Two days after oviposition we replaced the sugar water with distilled water for 24 or 48 h to increase the likelihood that each individual would feed on the blood host (B.D.R.). After 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 about 3 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. Randomly selected individuals were then placed in the distal end of 3.5cm/i.d. × 30 cm liter plexiglass cylinders. The distal ends of cylinders were covered with screen and a cardboard hatch was slid into a groove in the tube 5 cm from the distal end, thereby maintaining the insects in a 25-cm containment area. We initiated each trial by placing the open proximal end of the plexiglass cylinder perpendicular to the forearm of B.D.R. We randomly determined, using a random number generator in JMP IN 4.0.4 (SAS Institute, 2001), whether the right or left arm would be used for each trial. One minute later, the hatch was removed and the mosquito was allowed to fly to B.D.R.'s arm. If the mosquito did not approach the arm within 5 min, another mosquito was chosen for the trial. An individual mosquito was not used more than once during the experiment ($n = 35$).

Individuals were allowed to feed for 0, 1, 2, 3, or 4 min as determined via a random number generator in JMP IN 4.0.4 (SAS Institute, 2001). We started timing when an individual was observed inserting her mouthparts in the forearm of B.D.R. As soon as the predetermined feeding period elapsed, E.M. approached the feeding mosquito with a size 00 paint brush and swept the brush at 1 cm/s along B.D.R's forearm so that the mosquito flew away from its feeding site. Flight through the tube was recorded via a Panasonic video camera (model 13087) and video camera recorder (model AG1950). A ruler placed in the video shot at the same distance from the camera as the middle of the cylinder allowed us to determine the distance traveled by the mosquitoes. Immediately after its flight, each mosquito was chilled and again weighed to determine weight gain from blood feeding.

We obtained flight data by tracing the observed flight path with an overhead marker on an acetate sheet placed over a Sony Trinitron 27″ television monitor screen (model KVTS27). A clear 15-cm ruler taped perpendicular on the

flight tube during video recording allowed us to calibrate the distance flown on the television screen to the actual distance flown. We determined flight path length by running a cartographer's wheel (Brunton "Classic" Analog Map Measurer) over the convoluted traced path. To accurately measure the flight path, the videotape was played back in slow motion (30 frames/s or 1 frame = 0.03 s). Marks were made on the trace at 1-s intervals to record the distance flown per second. We then evaluated the consequence of mosquito mass change on parameters associated with escape (i.e., flight speed and flight path tortuosity).

Once the trace for each mosquito was analyzed, we divided the total distance flown by each mosquito by the total time it took the mosquito to fly this distance to calculate mean velocity. We used speed along the actual flight path, rather than linear displacement, because escape is not necessarily determined by distance from the host but rather by speed of movement of the mosquito (i.e., being hard to swat or capture), regardless of distance from the host. To measure flight path tortuosity, we traced the flight path with a ruler and examined the path for the presence of inflection points (a calculus-based technique of analyzing a line to detect points at which a line changes from being concave up to concave down, or vice versa). Using inflection points allows one to objectively define a turn. In addition, once the first inflection point is found, the tangent between inflection points is used to accurately determine turning angles.

Statistical analysis

We determined which variables were significantly correlated with mosquito velocity (cm/s), number of turns, and mean overall turning angle of fleeing mosquitoes by conducting three stepwise, mixed-model multiple regressions using whole effects for nominal and ordinal variables. For each stepwise regression we used (1) day of testing (1 vs. 2 vs. 3), (2) sequence on day of testing (1–22), (3) date of adult emergence (group 1 [emerged June 28, 29] vs. group 2 [emerged June 30, July 1, 2, 3, 4]), (4) days from last blood feeding (6–11), (5) days from last sugar water feeding (-2), (6) arm of experimenter fed upon (left vs. right), (7) initial weight of mosquito (0.60–1.87 mg), and (8) proportional increase in mosquito weight after feeding (-0.086–2.11), as independent variables. Some mosquitoes had a negative proportional increase in weight, as they were allowed to insert their mouthparts into B.D.R.'s arm, but not blood-feed, resulting in a net negative weight loss from the initial weighing to the end of the trial. All regression analyses were conducted using JMP IN 4.0.4 (SAS Institute, 2001).

Experiment 2: do mosquito mass changes alter chance of escape from hunting predators?

Cohorts of once-fed mosquitoes were again reared as in experiment 1. Two days after mosquitoes oviposited, we removed the sugar water from their cages for 24 h. After this deprivation period B.D.R.'s arm was placed in the rearing cage. As mosquitoes arrived, mosquitoes were placed individually into glass vials until six individuals were collected, at which point B.D.R. withdrew his arm. During the selection process, no individual was allowed to probe for more than 1–2 s.

After 6 females were collected, we flipped a coin to determine which three individuals would be fed and which would be sham fed. Feeding individuals were placed with the open end of their vials perpendicular to B.D.R.'s forearm. Sham feeders were also placed against B.D.R.'s forearm, but a cardboard hatch prevented them from inserting their proboscis and blood feeding. Three and one half minutes after mosquitoes initiated feeding, we removed the barrier

under the sham-feed vials and allowed those individuals to contact B.D.R.'s forearm for 30 s. This protocol ensured that both feeding and nonfeeding mosquitoes had experience with the host (i.e., exposure to similar odors, heat, humidity, etc.). At that point another series of coin flips determined which four individuals (i.e., two from each group: feeders vs. nonfeeders) would be exposed to spiders. We chose to select four mosquitoes out of the original six that fed on B.D.R.'s forearm, to ensure that the mosquitoes were randomly selected from within the different treatment groups (fed vs. unfed).

An individual zebra spider, which had been starved for 72 h, was placed in its own Petri dish (15 mm high × 60 mm i.d.) on the bottom of a 15 × 15 × 15-cm plexiglass cage. Spiders were allowed to habituate to the surroundings for at least 2 min. We then released each of the chosen mosquitoes into the cage. One minute later, we released the spider from its Petri dish by removing the lid. If a spider did not leave the dish within 1 min, it was immediately replaced. We observed the spider foraging for prey and terminated the trial when a mosquito was captured or 30 min elapsed without a capture. No mosquito or spider was used more than once in the experiment.

Frequently, we were unable to identify individual mosquitoes but could easily distinguish between fed and unfed individuals. Thus, the number of attacks were summed for the fed mosquitoes and also the unfed mosquitoes; resulting in a single data entry for preattack unfed, postattack unfed, preattack fed, and postattack unfed for each trial. This entire procedure was then replicated 42 times.

Statistical analysis
We determined whether mosquitoes that had been allowed to blood feed versus not to blood feed were more likely to escape, both before contact (i.e., as a spider was approaching and ready to pounce) and postcontact (i.e., after a spider had pounced at an individual) from jumping spiders. Because all variables were assessed simultaneously (i.e., unfed precontact vs. unfed postcontact vs. fed precontact vs. fed postcontact number of escapes), there is a lack of independence between the variables. This lack of independence means that neither a simple ANOVA nor survival analysis could be applied to these data. Instead, we used a randomized block ANOVA, essentially a paired *t* test with more than two groups (Zar, 1984). To accomplish this, for each trial, the number of escapes in each category was transformed into the arcsin proportion of attacks resulting in successful escapes. Post-hoc analysis was performed using pairwise contrasts for a randomized block design. We also determined if fed mosquitoes were actually captured more often than unfed mosquitoes using chi-square analysis. We then determined whether fed mosquitoes were captured in a shorter amount of time than unfed mosquitoes, using an independent samples *t* test. All analyses were conducted using JMP IN 4.0.4 (SAS Institute, 2001).

RESULTS

In experiment 1, the only variable reliably predicting the velocity of fleeing mosquitoes was proportional increase in weight (coefficient = −1.67, $F_{1,31}$ = 5.94, p = .021; Figure 1), as mosquitoes with a larger change in mass dispersed at a slower rate than mosquitoes that were less full of blood. Day of testing was nonsignificant (coefficient day 2–1 = 3.36, coefficient day 3–2 = −2.10, $F_{2,31}$ = 2.94, p = .068), although mosquitoes tended to disperse somewhat faster in later testing days (whole model; $F_{3,31}$ = 3.44, p = .029, r^2 = .18; Table 1).

Turning rate by fleeing mosquitoes was reliably predicted by initial weight of mosquito (coefficient = 11.93, $F_{1,31}$ = 7.78, p = .0089), as larger mosquitoes tended to make more turns.
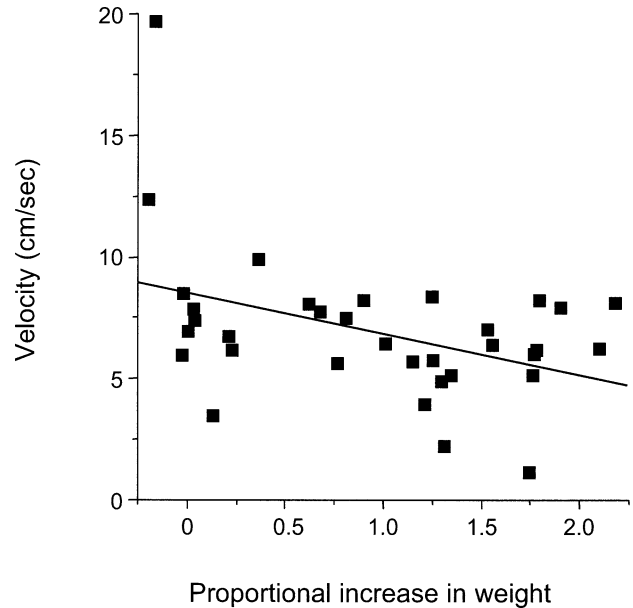

Figure 1
Proportional weight gain from blood feeding and velocity in female *Anopheles gambiae* mosquitoes (coefficient = −1.67, $F_{1,31}$ = 5.94, p = .021), after accounting for other significant variables using stepwise multiple regression (whole model; $F_{3,34}$ = 3.44, p = .029, r^2 = .18).

Not correlated with turning rate, but still contributing to the fit of the regression model, was proportional increase in weight (coefficient = −3.00, $F_{1,31}$ = 2.99, p = .094) and arm of experimenter fed upon (left vs. right; coefficient = −1.99, $F_{1,31}$ = 2.41, p = .13; whole model; $F_{3,31}$ = 4.45, p = .010, r^2 = .23). Mean turning angles were well predicted by the amount of time since the last blood meal (coefficient = 14.43, $F_{1,32}$ = 6.51, p = .016), as mosquitoes that had been deprived of blood for a longer period of time made sharper turns than did mosquitoes with more recent blood meals. Arm of experimenter was not significant (coefficient = 19.75, $F_{1,32}$ = 2.55, p = .12), but still contributed to the overall fit of the regression model (whole model; $F_{2,32}$ = 6.00, p = .0061, r^2 = 0.23; Table 1).

In Experiment 2, in 25 of the 42 replicates at least 1 mosquito successfully escaped the foraging spider, and in 33 of the 42 replicates spiders were able to capture a mosquito within 30 min. Analysis of the escape data showed that unfed pairs of mosquitoes had different chances of evading the spider predators ($F_{3,72}$ = 3.34, p = .024; Figure 2). Unfed mosquitoes were more likely to escape from jumping spiders as the spider approached than were fed mosquitoes. Once a spider pounced at a mosquito, however, it escaped a similar number of times whether fed or unfed. Due to the size of the small cage, however, there was no difference in the overall number of fed versus unfed mosquitoes (19 vs. 14) that were captured by spiders (χ^2_1 = 0.76, p = .38). Furthermore, fed mosquitoes were not captured faster compared to unfed mosquitoes (mean ± SE; fed vs. unfed; 379 ± 77 vs. 358 ± 89 s; t_{31} = 0.18, p = .86).

DISCUSSION

Our experiments provide strong experimental evidence that mosquitoes incur substantial risk due to increased mass from blood feeding. Such risk occurs both at the feeding site as well as at postfeeding sites. Thus, while blood feeding is in general

Table 1

Variables included in the stepwise multiple regressions to predict velocity, number of turns, and mean turning angle by blood-feeding mosquitoes dispersing away from a human host

Variable	Velocity (cm/sec)		No. of turns		Mean turning angle	
	$F_{1,31}$	p	$F_{1,31}$	p	$F_{1,32}$	p
Day of testing (1 vs. 2 vs. 3)	2.94[a]	.068[b]	1.14[a]	.34	0.25[c]	.78
Sequence on day of testing (1 to 22)	0.69	.41	.75	.39	.58	.45
Date of adult emergence (group 1 vs. group 2)	0.24	.63	0.97	.33	0.00	.99
Days from last blood feeding (6 to 11)	0.24	.63	0.18	.68	6.51	.016*
Days from last sugar water feeding (1–2)	0.24	.63	0.11	.74	0.40	.53
Arm of experimenter fed upon (left vs. right)	0.76	.39	2.41	.13[b]	2.55	.12[b]
Initial weight of mosquito (0.60–1.87 mg)	0.93	.34	7.78	.0089*	0.86	.36
Proportional increase in mosquito weight after feeding (−0.086–2.11)	5.94	.021*	2.99	.094[b]	0.18	.68

* Variable was significant in the overall stepwise regression model, $p < .05$.
[a] df = 2,31.
[b] Variable contributed to the overall stepwise regression model, though not statistically significant ($p < .05$).
[c] df = 2,32.

a dangerous activity, the degree of danger increases as a function of the volume of blood imbibed.

The risk of death associated with blood feeding in mosquitoes is best evaluated in terms of the trade-off between fecundity and survivorship that is mediated via blood feeding. Fecundity increases as a function of blood meal size (Briegel, 1990) but so, too, should mortality, both at the host and after host feeding. This trade-off is asymmetric in two ways. First, mosquitoes should be able to accurately assess the aforementioned deterministic blood volume–fecundity curve, whereas the likelihood of escape is a complex variable that depends on a number of probabilistic parameters including host position, reaction speed, physiological state, etc. Therefore, the

Figure 2
Unfed female *Anopheles gambiae* mosquitoes escape more often from foraging jumping spiders versus blood engorged females ($F_{3,72}$ = 3.34, $p = .024$), when examining two phases of spider search, prepounce (approaches prey [PRE-]) and postpounce (tackles prey [POST-]). Columns with different letters are significantly different at $p < .05$, using pairwise contrasts for a randomized block ANOVA on arcsin-transformed data. The columns show results from non-transformed data.

probability of escape and resulting survivorship cannot be accurately estimated as a function of mass gain. Second, it has been suggested that the blood meal–fecundity function positively decelerates (see Anderson and Roitberg, 1999), while the blood meal–mortality curve appears to increase linearly. If true, mosquitoes should be increasingly willing to abandon hosts as (1) their mass increases and/or (2) their hosts show signs of being disturbed. However, controlled experiments have not been conducted to test this prediction.

One possible criticism of this experiment is the use of diurnal spiders and crepuscular mosquitoes under daylight conditions. In defense of this approach, we note that salticid spiders are known to attack mosquitoes in nature (Foster W, personal communication), more particularly in Africa where *A. gambiae* naturally occurs. With regard to light conditions, Canyon and Hii (1997) found similar rates of predation by geckos on *Aedes aegypti* during light versus dark conditions. From a biological perspective, resting mosquitoes must deal with predators under both diurnal and nocturnal conditions.

In contrast to our findings, Canyon and Hii (1997) found that unfed mosquitoes were more likely to be preyed upon by geckos than were their fed counterparts, possibly due to differences in activity level (i.e., the more active mosquitoes were more apparent to the geckos). Canyon and Hii (1997) confined their test animals to a small terrarium where geckos could track actively escaping mosquitoes because they could not disperse. We observed a similar tracking phenomenon in our test and argue that this effect would disappear in larger environments (e.g., human abodes). Thus, the important index of predator evasion is the frequency of escapes, which clearly favored unfed individuals.

There is some question whether engorged mosquitoes displayed reduced flight speed and agility due to physical impediment from mass change or whether they choose to alter their escape behavior. We suggest that an engorged female has little or no reason to return to its host and if anything should be more willing to expend effort to escape because of the small marginal returns from more blood. On the other hand, our results show that the longer a mosquito went without feeding, the more likely it was to turn back toward its host after initial escape. The latter results indicate that flight paths are plastic and that this flexibility is in the direction predicted by optimal escape theory (Lima and Dill, 1990).

In conclusion, mass change associated with acquisition of nutrients can have profound effects on organisms whose
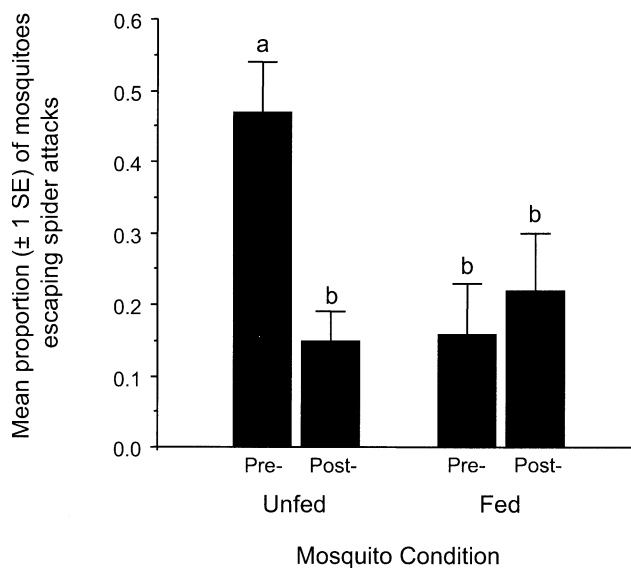
primary mode of transport is flight. This effect crosses taxonomic boundaries from birds (e.g., robins) to insects (e.g., potato beetles; MacQuarrie, 2002). Further research is required to determine if mass change is also important for ground-based organisms that rely on speed and agility to evade predators.

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