

TESTS OF *SABETHES CYANEUS* LEG PADDLE FUNCTION IN MATING AND FLIGHT

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ABSTRACT. Both sexes of the mosquito *Sabethes cyaneus* have conspicuous midleg paddles of unknown function. These paddles are displayed prominently by males during courtship. However, we found that paddle removal reduced successful mating of females, not males. Males without paddles approached and courted females normally, but females without paddles were rarely approached by males. Paddle removal appeared not to alter flight, and paddleless females oviposited in the manner characteristic of *Sabethes*, suggesting that the paddles do not aid in hovering or rapid reverse flight. We conclude that paddles of females are used in attracting males but that their function in males remains undetermined.

Both sexes of *Sabethes* (*Sabethes*) *cyaneus* (Fabricius), and other species in the subgenus *Sabethes*, have conspicuously broad iridescent paddles on the midlegs, formed from elongate scales on the tibia and first and second tarsomeres. The function of these paddles is not known. Gillett (1972) speculated that they might serve in locomotion over water surfaces or as "sex-signal receivers." *Sabethes cyaneus* mates at its preferred resting sites on the underside of horizontal sticks and vines, where the male performs an elaborate courtship while facing the female (Hancock et al. 1990). Because *Sa. cyaneus* males perform several striking stereotyped waving and wagging movements involving the paddled midlegs during courtship, we tested the hypothesis that paddles are critical to mating success by removing them from experimental subjects. These came from a large colony derived from specimens collected in eastern Panama in 1983 by J. L. Petersen, Gorgas Memorial Laboratory, Panama.

To measure the influence of male and female paddles on successful mating, we separated the sexes <24 h after emergence, before mating behavior occurs, and removed the paddles from about half of each sex 15–23 days later. These mosquitoes were anaesthetized with diethyl ether, mired on a wet glass slide, and paddle scales removed with a blade or brush. Specimens with intact paddles, which served as treated controls, were anesthetized and manipulated in the same manner. After 24 h, the 4 treated groups and one untreated group were combined with similar-size groups of untreated specimens of the opposite sex (19 males, 20–23 females). Each combination was housed in a 38-liter clear acrylic cage containing water wicks, diluted honey on sponges and a horizontal wooden stick. To keep male numbers constant, we checked

cages every 12 h and replaced dead males with reserves of the appropriate treatment. Direct observations of courtship were made occasionally. After 72 h, spermathecae of surviving females were checked for sperm. Two replicate experiments were conducted, using mosquitoes from different cohorts.

Removal of paddles from males did not appear to alter either their mating behavior or the response of females to their courtship. The insemination rate of normal females by paddleless males (79%) showed no significant differences from 3 controls (Table 1). However, paddleless females housed with normal males had significantly lower insemination: 8% ($P < 0.001$).

Male paddles clearly were not essential to successful courtship, culminating in insemination, whereas female paddles were essential. It remains possible that females accepted paddleless males at a lower rate than normal males and that 72 h nevertheless had been sufficient time for both kinds of males to inseminate most females. The striking failure of most paddleless females to become inseminated in 72 h suggested 2 possible causes: the male or female might abort courtship, or the male might decline to initiate it. The latter seemed particularly likely, because males typically patrol sticks where both sexes rest. A male approaches a resting target mosquito, hovers beside it, then attempts to align himself opposite the target by grasping its wings with one midleg, then pivoting to face the target. If the male does not attempt or achieve alignment, courtship is not initiated and insemination does not occur (Hancock et al. 1990).

To test whether males were attracted to paddleless mosquitoes, we measured the attempted alignment frequency of normal and paddleless males toward normal and paddleless targets of each sex. They were prepared as above at >10 days after emergence and allowed 1–4 days for recovery. Then 10 specimens of each of the 4 types, drawn from pools of each of the 4 treatment groups prepared from one cohort, were placed together twice, 2 days apart, for 2-h ob-

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Table 1. Insemination rates of normal (paddle-intact) and paddleless females after 72-h exposure to normal and paddleless males.

Sex	Paddle treatment combinations									
	1		2		3		4		5	
	Removed Intact		Intact ¹ Intact		Intact Intact		Intact Removed		Intact Intact ¹	
Replicate	1	2	1	2	1	2	1	2	1	2
Sample size ²										
Initial	20	20	23	20	20	21	20	20	22	21
Final	19	19	22	15	20	21	9	16	20	21
Inseminated (%)	16	14	14	9	19	15	0	2	19	17
	(84)	(74)	(64)	(60)	(95)	(71)	(0)	(13)	(95)	(81)
Total % inseminated ³	79ab		62b		83a		8c		88a	

¹ Specimens with intact paddles, anesthetized with diethyl ether and manipulated on slide.

² All replicates contained 19 males, maintained by replacement of dead males.

³ Values followed by same letter were not significantly different ($P > 0.05$) according to G -tests of independence. Combination 2 was significantly lower than combinations 3 and 5 (respectively: $G = 7.1$, $df = 1$, $P < 0.01$; $G = 4.3$, $df = 1$, $P < 0.05$), but combination 4 was significantly lower than all others, including combination 2 ($G = 10.2$, $df = 1$, $P < 0.005$). Analysis of arcsine-transformed values by orthogonal contrast ANOVA (Sokal and Rohlf 1981) detected a significant difference only between combination 4 and the others (e.g., contrast of combinations 2 and 4: $F = 42.0$; $df = 1, 5$; $P < 0.01$).

Table 2. Frequency of attempted mating alignments by normal (paddle-intact) and paddleless males with each other and with normal and paddleless females (10 of each of 4 types), replicates combined.

Paddle status of aligning male	Sex and paddle status of target mosquito					
	Female			Male		
	Intact	Removed	G -test ¹	Intact	Removed	G -test ¹
Intact	109 (98%)	2 (2%)	132.6*	71 (100%)	0 (0%)	97.1*
Removed	125 (91%)	13 (9%)	104.8*	90 (98%)	2 (2%)	108.3*

¹ Goodness-of-fit G -tests: *, $P < 0.001$.

servation periods, in one 86-liter acrylic cage with a horizontal stick. They were returned to their respective pools between observation periods. Attempted alignments involving 8 possible combinations of mosquito types (2 types of males attempting alignment with 4 types of targets) were recorded as they occurred, whether or not stationary alignment was achieved. Aligned pairs were broken up immediately, to prevent courtship. Two replicate experiments were conducted (8 h total observation time), involving different cohorts of mosquitoes.

Frequencies of attempted alignments with normal targets were much higher (96% of all alignments) than with paddleless targets, regardless of condition of the aligning male and the sex of the target (Table 2). Both intact and paddleless males attempted significantly more alignments with females (61 and 60%, respectively) than with males (intact: $G = 8.8$, $df = 1$, $P < 0.005$; paddleless: $G = 9.2$, $df = 1$, $P < 0.005$). The high frequency of male attraction to males suggests that prior to alignment sexual discrimination is poor. The moderately more frequent attraction to females may reflect an operational sex ratio distortion intrinsic to the experimental

design, because the approaching male himself and other patrolling males were an unavailable part of the target-male pool. The difference in frequency of total alignment attempts by paddleless males (56%) and normal males (44%) was significant ($G = 5.6$, $df = 1$, $P < 0.05$), but its meaning is unclear.

Thus, female paddles (and incidentally, male paddles) are apparently important for attraction of males. They may aid in species recognition and possibly enhance visibility, because they strongly reflect ultraviolet light (T. Eisner, personal communication); whether the males perceive this color is unknown. The attractiveness of the paddles is sufficient to explain the low insemination rate of paddleless females in the previous experiment. However, this explanation does not address paddle function in males.

Gillett's (1972) suggestion that *Sabethes* paddles are used to tread water seems unlikely. The scales are still recumbent when newly emerged adults in the laboratory crawl off the water's surface. The natural sites of *Sa. cyaneus* larval development are accumulations of water within tree cavities accessible through small openings (Galindo et al. 1951). After emergence, adults

apparently never enter these tree holes; females oviposit by shooting eggs into the openings while hovering. It seemed to us more likely that the paddles serve as airfoils during flight. Conceivably they help to maintain the stable, hovering flight characteristic of both sexes, being held horizontally as the mosquito approaches potential mates, hosts, and resting and oviposition sites. They might also be used when the mosquito darts backward in response to sudden movement or loud noise, or when laying eggs, because the paddles are thrust forward or downward at these times. During oviposition in the laboratory, the female hovers over the opening in an oviposition cup, and at the instant of paddle thrust she propels an egg into it with a flick of the abdomen, immediately flying upward and backward to 1–4 cm above the original hovering plane.

To test whether paddles are instrumental in hovering and reverse flight during oviposition, we removed them from 20 females and manipulated another 20 controls, allowed all to take a replete blood meal, then held them 1 week to allow egg maturation. During one 6-h period we placed oviposition cups into cages of normal and paddleless females and made 301 clear video recordings of ovipositions of the former group and 139 of the latter; others were observed directly.

We detected no consistent differences between the oviposition behavior of paddleless and normal females, either directly or by normal-speed and frame-by-frame video analysis of the ovipositions recorded. Paddleless females appeared to hover as smoothly and dart upward as rapidly (about 0.08 sec) as normal females, shaved and paddled midlegs being thrust downward similarly. Therefore, paddles seem unimportant in hovering and reverse flight. This is supported by observations on *Sabethes* (*Sabeth-*

oides) *chloropterus* (Von Humboldt) made by Galindo (1957) and recently by us. This species lacks paddles, oviposits as *Sa. cyaneus* does and can dart backward when disturbed.

Thus, the function of the paddles remains enigmatic. Their sex-limited role in female attractiveness seems likely to have evolved secondarily. If male paddles sometimes are used by females when evaluating mates during courtship, they are certainly not critical to successful insemination. A study of the frequency of mating success of paddleless males remains to be done. Also, there is a need to test the possibility that paddles are used to detect physical stimuli or to create multiple strike targets that confuse predators or deflect attack.

We are indebted to J. L. Petersen for establishing the original colony of *Sa. cyaneus*, to L. E. Munstermann for providing the founding individuals for our colony and to R. M. Taylor for provoking us to conduct the experiments.

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