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Mosquitoes: Biting Behavior Inhibited by Ecdysone

Abstract. *Biting in* Anopheles freeborni *is inhibited during ovarian development. Biting inhibition is triggered by ecdysone, a hormone produced by the ovary during oogenesis. Biting inhibition does not occur in females after the removal of ovaries, but is restored by replacing ovaries or injecting ecdysone. Ecdysone also inhibits biting behavior when it is fed to females. This is the first example of ecdysone controlling a nonmolt-related behavior in insects.*

Most mosquitoes must acquire a blood meal in order for their eggs to mature, and in the process of obtaining the blood meal they often transmit disease to man. Between each cycle of egg production the females bite aggressively. After the mosquitoes have become engorged with blood they cease biting until oogenesis is complete (*1*). Therefore, the phase of egg development occurring after the blood meal coincides with a period of nonbiting behavior. The data in this report demonstrate that the association between egg production and biting behavior is mediated by the ovarian hormone ecdysone.

The ovaries begin secreting ecdysone shortly after the female takes a blood meal (*2*), so that the ecdysone titer is increased at the same time that biting behavior disappears. Experiments were conducted with 2-day-old female *Anopheles freeborni*. The ovaries were surgically removed according to a modification of the technique of Spielman (*3*). After a 24-hour recovery period these females were placed in a cage with an excess of males to facilitate mating. Two days later, the same females were allowed to become engorged with blood by feeding from my hand. A group of females with sham operations and a group of normal females were allowed to feed in the same way. After the initial feeding period, nonfeeders were removed so that each group consisted of females which had fed to repletion. On subsequent days each group was given the opportunity to feed for 10 minutes, and the number that fed was recorded. During the experi-

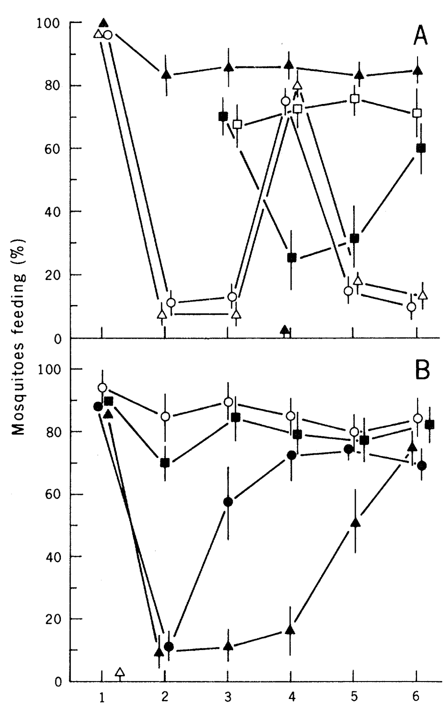

Fig. 1. (A) The blood-feeding behavior of *A. freeborni*. Each point represents the response of 25 females: (▲) ovariectomized, (△) with sham operations, and (○) normal. Early on day 3, one-half of the ovariectomized females were implanted with an undeveloped ovary from a 2-day-old donor and subsequently assayed for feeding behavior (■). Control females were implanted with pieces of midgut (□). Vertical bars show the standard error of the mean for three experiments. The arrow indicates the time at which females with sham operations and normal females oviposited. The mosquitoes were maintained at 26°C, with a photoperiodic cycle (L : D) of 15 hours of light and 9 hours of darkness. (B) Females were injected with β-ecdysone at three different concentrations: (▲) 5 $\mu g/\mu l$, (●) 1 $\mu g/\mu l$, (■) 0.5 $\mu g/\mu l$, or (○) with saline. The open arrow indicates the time of injection. Each point represents the mean response of 25 females; vertical bars show the standard error of the mean for three experiments conducted at 26°C, L : D, 15 : 9.

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ment, females were allowed to lay eggs at will.

The difference in biting behavior of the three groups is shown in Fig. 1A. At least 80 percent of the ovariectomized females fed each day, whereas less than 20 percent of the females with sham operations and the normal females fed on days 2, 3, 5, and 6. Eighty percent of the females with sham operations and the normal control females completed egg development and oviposited on day 4 of the experiment and then took a second blood meal. After becoming engorged for the second time, these females entered a second egg maturation cycle, such cycles being characterized by the disappearance of biting behavior on days 5 and 6. Early on day 3, ovariectomized females, which had taken blood on days 1 and 2, were implanted with an ovary from donors that had not received a blood meal. After a 20-hour recovery period these females with implanted ovaries were again allowed to feed for 10 minutes; 70 percent of them took blood. In most of these females (N = 29) the implanted ovary began to develop eggs and the patterns of biting behavior changed to that of normal females (Fig. 1A); that is, fewer than 30 percent took blood on the 2 days following the blood meal. Biting behavior reappeared in the females with implanted ovaries on day 6, when 61 percent of them took blood. Ovariectomized females that were implanted with pieces of midgut were used as controls, and at least 65 percent of these mosquitoes continued to bite each day during the course of the experiment. These results indicated that the disappearance of biting behavior after a blood meal was due to an endocrine effect originating from the developing ovary.

To verify that it was ecdysone, and not some other ovarian hormone, that was affecting biting behavior, I injected (*4*) various concentrations of β-ecdysone (*5*), 1 μl per injection, into a group of ovariectomized females. Control females were injected with the same amount of solvent without β-ecdysone. Both groups were then exposed to my hand for 10 minutes daily and scored for blood feeding behavior. The results (Fig. 1B) indicated that the biting drive, characteristic of ovariectomized females, could be abolished by injection of ecdysone. The duration of biting inhibition depended on the amount of ecdysone injected. Ecdysone at a concentration of 0.5 μg/μl inhibited biting behavior in only 20 percent of the females tested at 20 hours after injection (day 2). Injections of 1 μg/μl were effective in inhibiting biting in 50 percent

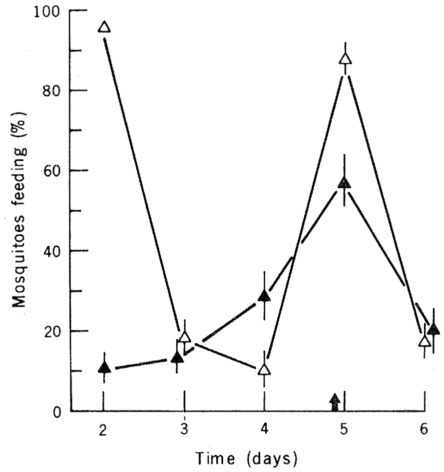

Fig. 2. The blood-feeding behavior of *A. freeborni* females injected on day 1 with (▲) β-ecdysone (5 μg/μl) or (△) saline. Each point represents the mean response of 50 females; vertical bars show the standard error of the mean for three experiments conducted at 26°C, L : D, 15 : 9. The arrow indicates the time when oviposition first occurred.

or more of the test population for approximately 2 days (days 2 and 3) after injection. Treatment with 5 μg/μl extended the nonfeeding period, as defined by the number of days when 50 percent or more of the test population did not take blood, to almost 4 days after injection. Females injected with a structurally related compound, cholesterol (*6*), showed biting behavior similar to that of the saline-injected controls.

Injections of ecdysone into females with intact ovaries also inhibited the biting drive. When such females were injected with β-ecdysone and subsequently assayed for biting behavior as in

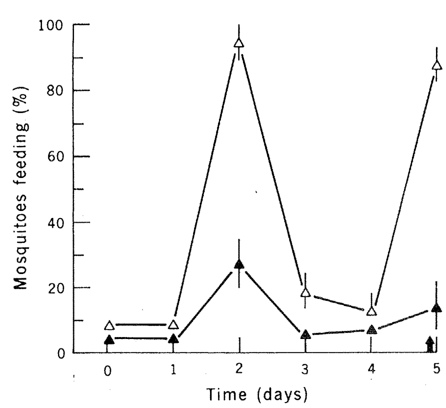

Fig. 3. The blood-feeding behavior of *A. freeborni* females fed from emergence (day 0) on (▲) 1-g sugar cubes impregnated with 10 mg of β-ecdysone or (△) similar sugar cubes impregnated with the ecdysone solvent. Each point represents the mean response of 75 females. Vertical bars show the standard error of the mean for two experiments conducted at 26°C, L : D, 15 : 9. The arrow shows the time at which oviposition occurred.

the preceding experiments, most of them did not feed until day 5 (Fig. 2), whereas control females, injected with saline, fed at the first opportunity on day 2. The pattern of biting behavior in the control females reflected a normal egg development cycle, with the number of bites decreasing on days 3 and 4, then increasing on day 5, when the majority of these females deposited eggs. After they became engorged on day 5, both the ecdysone-injected and control females began a second egg maturation cycle, characterized by reduced biting levels on day 6.

Mosquitoes that ingested β-ecdysone also showed suppressed biting behavior. Females given free access from the time of emergence to sugar cubes impregnated with β-ecdysone (*7*) showed a marked reduction in biting behavior when compared with sugar-fed controls (Fig. 3). In this experiment neither the experimental nor control insects fed at the first feeding opportunity on day 1. However, on day 2, 93 percent of the control females became engorged with blood and then entered the first egg maturation cycle during which biting behavior disappeared (days 3 and 4). On day 5 the controls oviposited, and 90 percent of them took a second blood meal. Oral administration of ecdysone damped this cyclic biting pattern associated with egg development. Only 25 percent of the ecdysone-fed females took blood on day 2 and even fewer (15 percent) fed on day 5.

In experiments with *Aedes aegypti*, Judson (*8*) showed that 75 percent of virgin females would continue to feed even after they had become engorged. To ensure that lack of insemination was not responsible for the continued biting behavior of the ovariectomized females in this study, I examined the spermatheca (the sperm storage organ) of each female for the presence of sperm. All females were found to be inseminated.

Ecdysone titers usually begin to increase between 10 and 15 hours after a blood meal (*2*). However, long before this time blood-engorged females are no longer attracted to a host (*9*). Although lack of attraction does not necessarily imply that biting behavior is inhibited, in my study less than 5 percent of the ovariectomized females assayed for biting behavior at 1, 5, and 10 hours after feeding attempted to take a second blood meal. Thus, while the lack of biting behavior observed at 24 hours after the blood meal is related to ecdysone, the immediate postfeeding inhibition of biting is probably due to other factors (*10*).

To date, studies on ecdysone in adult

mosquitoes have been centered on its role in egg development. However, behavioral roles for ecdysone are known in other insects. In the desert locust, *Schistocerca gregaria*, the rise in ecdysone titer which occurs in conjunction with molting also acts on the central nervous system of the animal to suppress postecdysis locomotor activity (*11*). Locusts in this state enter a solitary phase thereby increasing the probability of successfully completing the molt. Whether ecdysone-induced inhibition of biting behavior is also characterized by changes in the central nervous system remains to be investigated.

Note added in proof: Klowden and Lea (*12*) have reported that an unidentified hemolymph-borne substance, present during egg development, inhibits host-seeking behavior in *Aedes aegypti*.

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4. Injections were made with glass needles fabricated from capillary tubing and calibrated.
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13 March 1979

Nest Guard Replacement in the Antarctic Fish *Harpagifer bispinis*: Possible Altruistic Behavior

Abstract. *Nesting biology of the Antarctic plunder fish,* Harpagifer bispinis *(Schneider), was examined at Arthur Harbor, Antarctic Peninsula, during the austral winter, 1975. Females prepare nest sites, spawn, and guard the eggs for 4 to 5 months, the longest guarded incubation period reported for any fish species. If this guard is removed, it is soon replaced by a conspecific, usually male. If the second guard is removed, a third replaces it. Guards are essential to ensure nest survival. Selfish or parental acts or acts of kinship or reciprocity do not adequately explain guard replacement. The act may be altruistic.*

True altruism occurs when an act is performed that benefits an unrelated individual to the detriment, defined in terms of personal fitness, of the donor who can expect neither immediate nor future repayment (*1–3*). To date, such acts have not been documented in natural populations, but debate surrounding their possibility remains active (*1, 4, 5*). Recent studies that have examined seemingly altruistic behavior in natural populations have indicated that the observed behavior could be explained without resort to true altruism (*3, 6*). Here I report on apparent altruistic acts in the nesting behavior of *Harpagifer bispinis*, a small demersal fish found in shallow, rubble bottom coves along the Antarctic Peninsula (*7*). Females prepare nest sites in June and spawn from late June to mid-August in Arthur Harbor (64°46′S, 64°04′W), where this study was conducted (*8*). If undisturbed, the female remains on the nest (Fig. 1) until the eggs hatch 4 to 5 months later. This is the longest brooding period reported for any fish (*9*) but may be common among Antarctic species (*10*). The guard is necessary for egg survival since it protects the nest from egg predators and prevents a fungal growth that destroys all unguarded nests within 2 weeks (*8*). If the initial guard is removed, a second fish, usually male, assumes most guard responsibilities. If the second guard is removed, a third fish, also male, assumes guardianship.

When apparent altruistic acts, such as the above, are observed, one of several alternative hypotheses is generally invoked: (i) the act is primarily selfish and the apparent altruism is incidental; (ii) the act is parental; (iii) the act is one of kinship (*1*) in support of a closely related individual; (iv) the act is one of reciprocity (*2*); (v) the act is misdirected selfish or parental behavior (*11*); or (vi) the act is one of true altruism. Each hypothesis was tested in turn, although the tests were limited by laboratory and field conditions, time, and the vagaries of Antarctic weather. I observed social interactions among *H. bispinis* on 48 dives in Arthur Harbor and for 150 hours over a 7-month period among a population maintained in a tank (95 by 50 by 50 cm) in the laboratory at Palmer Station. Behavioral observations in the field were limited because fishes tend to conceal themselves under rocks and because observation dives were limited to 30 minutes. Nevertheless, activities of individual fishes could be observed on successive dives since each individual has a distinct color pattern. As a result of a series of five dives conducted between 15 August and 27 December 1975 in a small cove (0.1 ha), approximately 30 individuals and seven nests were observed. The seven nest guards remained on their respective nests throughout the observation period. The other fish roamed about the cove but were never observed on the mud bottom area that separated the cove from other rubble areas. Of the 27 fish observed on the initial dive, 18 were recorded on the final dive. Five of the 12 fish observed on subsequent dives were also present on the final dive. The greatest straight line distance traveled by an individual still in the study area was 15 m. Protected sites, namely crevices and crannies under and between rocks, were abundant in the cove.

In order to make more extensive observations and manipulations, eight fish were introduced into the laboratory tank, which had only four protected sites. Initially, a weak dominance was established in which two coequal males tended to dominate the other individuals during encounters involving food capture. Dominance with respect to site use was also observed on occasion. All fish tended to rove; no individual occupied a single site for more than 24 consecutive hours, and site sharing by three to five individuals was not uncommon. On 10 August, one female spawned and began to guard the resulting nest. Observations continued until 15 December.

The following six points must be considered.

1) Selfish acts increase the fitness of the individual performing the act without regard to possible secondary effects, either beneficial or detrimental, to another individual. If guard replacement were a selfish act, the benefit accrued must exceed the cost involved. In the field, no nest guard was displaced from its nest by another fish. These fish may benefit by obtaining a permanent protected site. However, any overhanging rock can

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