

ENDOGENOUS REGULATION OF THE ATTRACTION OF *Aedes Aegypti* MOSQUITOES

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ABSTRACT. Even when host attractants are present, there are times when endogenous physiological mechanisms prevent female mosquitoes from responding to them and engaging in host-seeking behavior. These times include a brief postemergence period, periods of the day determined by a circadian clock, and following a blood meal before and after eggs develop, which are controlled by nervous and humoral mechanisms. Other factors such as age, nutrition, and mating status can further modulate host-seeking behavior. The interplay of these factors affects the frequency at which mosquitoes will approach a host and feed on its blood, thus affecting the rates of parasite acquisition and transmission.

INTRODUCTION

Although we tend to consider mosquitoes as the sharks of the insect world, relentless in their pursuit of a meal of blood, they are able to regulate their behavior and engage in host seeking only at times that are biologically appropriate. A good example of this endogenous behavioral regulation is the rhythmicity that limits the activity of many species to a species-specific circadian window, even though host stimuli may be present at other times. However, even during this circadian window, host-seeking behavior may not occur although attractants are present. It is often suppressed after the mosquitoes have ingested blood and their eggs have begun to develop, which in theory limits host-seeking behavior to the beginning of each gonotrophic cycle. We see evidence of this behavioral inhibition when collections using attractant traps are compared with those from nonattractant sweep net collections; the former usually yield few gravid mosquitoes (Bidlingmayer 1974).

This behavioral inhibition following blood ingestion has played a major role in the development of our concept of the mosquito gonotrophic cycle, in which the relationship between reproduction and hematophagy has been used to estimate the age composition and feeding frequency of mosquito populations. It is commonly assumed that anautogenous mosquitoes will seek a host, ingest its blood, develop a batch of eggs, and begin host seeking again after those eggs are laid. We accept this assumption because it agrees with our observations of the behavior of mosquitoes in the laboratory. Laboratory colonies are encouraged to mate, are routinely maintained on carbohydrate, are allowed to bloodfeed on an anesthetized host, and then allowed to oviposit a few days later. When these populations grow older, they are discarded and replaced with a younger population. However, in the field, where hosts are not anesthetized, carbohydrate is not always readily available, if females do mate they

mate with a male raised on the same marginal nutritional regimen, and older females are the principal vectors, the gonotrophic cycle may differ from what we observe in laboratory populations. Indeed, there are many examples of field populations of mosquitoes containing serologically mixed blood meals, or gravid females that have been recovered from biting collections (Gould et al. 1970, Mitchell and Millian 1981, Ramaiah and Das 1992) that attest to the necessity of reevaluating the assumptions upon which the traditional gonotrophic cycle is based. Central to our understanding of the conditions under which a mosquito may feed multiply and violate these assumptions is an understanding of the mechanisms that regulate mosquito host seeking.

REGULATION OF HOST-SEEKING BEHAVIOR

Regulation of behavior after adult emergence: When mosquitoes are reared under suboptimal conditions as larvae, they often fail to attain their potential maximum adult size (Wada 1965), and at least in the laboratory, a significant proportion of these small adults also fail to engage in host-seeking behavior (Terzian and Stahler 1949, Klowden et al. 1988). Of those mosquitoes that do seek a host, 2 blood meals may be necessary before a batch of eggs can be matured (Feinsod and Spielman 1980). Nasci (1986a, 1986b) has shown that in the field, smaller mosquitoes are not well represented in bloodfed populations, and has suggested that these smaller adults are not as successful in obtaining a reproductive meal as are larger females.

Following adult emergence, there is usually a period of one to 2 days during which host-seeking behavior fails to be expressed. During this time, the corpora allata produce juvenile hormone that stimulates the ovaries to develop to the resting stage (Gwadz and Spielman 1973) and makes

several other tissues competent for the later production of vitellogenin (Flanagan and Hagedorn 1977, Rossignol et al. 1982). Allatectomy of *Culex* mosquitoes at emergence not only blocks egg development, but also the development of post-emergence biting behavior (Meola and Petralia 1980). Allatectomy after the first blood meal also prevents the development of a 2nd biting cycle (Meola and Readio 1987). The restoration of biting behavior when juvenile hormone is applied or active corpora allata are implanted suggests that juvenile hormone induces biting in *Culex*, although this mechanism was not evident in species of either *Anopheles* or *Aedes* (Bowen and Davis 1989). The reasons for the differences between *Culex* and these other genera in the regulation of biting are not understood (Meola and Readio 1988).

Regulation of behavior by an endogenous circadian clock: The expressions of many mosquito behaviors are governed by an endogenous circadian clock. Mosquitoes are often most active at dusk and dawn (Senior White 1953, Haddow 1964, Corbet 1966, Taylor et al. 1979, Nelson et al. 1978). Their flight activity and subsequent host-seeking behavior are controlled by a circadian clock (Jones 1982, Clopton 1984), but are modified by blood ingestion and insemination (Jones and Gubbins 1978, Rowland 1989). Behaviors that are expressed during the windows of circadian activity can also be influenced by the number of gonotrophic cycles the female has undergone (Crans et al. 1976, Aslam et al. 1977, Charlwood et al. 1986) and whether she is infected with parasites (Rowland and Lindsay 1986, Rowland and Boersma 1988).

Regulation of host-seeking behavior after bloodfeeding: At least for *Aedes aegypti* (Linn.) host seeking appears to be the "default" behavior and is expressed whenever host stimuli are received, unless endogenous factors within the mosquito inhibit its expression. Two overlapping mechanisms of distention-induced inhibition and oocyte-induced inhibition prevent mosquitoes that have been maintained under ideal conditions from seeking another host until their eggs are laid (Klowden and Lea 1978, 1979a, 1979b; Klowden 1981). These 2 mechanisms are primarily responsible for the restriction of host-seeking behavior to the beginning of each gonotrophic cycle.

Immediately after a large blood meal, abdominal distention from the blood inhibits host-seeking behavior as long as the blood volume remains above a certain threshold (Klowden and Lea 1978). Stretch receptors appear to maintain this distention-induced inhibition until the meal has been metabolized and excreted (Klowden and Lea 1979b). Mosquitoes that ingest partial meals

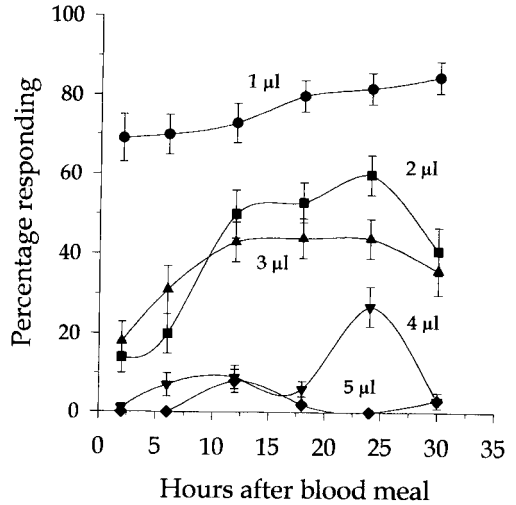


Fig. 1. Percentage of mosquitoes responding to host stimuli in an olfactometer after receiving graded volumes of blood.

that are below the threshold continue to seek a host until that threshold has been reached. For example, the data in Fig. 1 represent the host-seeking behavior of *Ae. aegypti* mosquitoes after they were given measured meals of blood. Host-seeking behavior declined with increasing meal size when the volumes exceeded the threshold. As the meal was digested and excreted, host seeking gradually returned, but then began to decline once again at 30 h as the eggs developed.

If the blood meal is large enough to initiate and sustain egg maturation, a 2nd mechanism overlaps and maintains the inhibition of host seeking that began with abdominal distention (Klowden and Lea 1979a). In *Ae. aegypti*, this inhibition begins at about 30 h after blood ingestion and lasts until oviposition occurs (Fig. 2). Unlike distention-induced inhibition that appears to be mediated by the nervous system, this oocyte-induced inhibition is initiated by a complex interplay between the ovaries, fat body, and neurosecretory cells (Klowden 1981, Klowden et al. 1987). A neuropeptide, *Aedes* Head Peptide I, which is released from neurosecretory cells in the brain and midgut, appears to be directly responsible for the behavioral inhibition during oogenesis (Brown et al. 1994). Nongravid mosquitoes that fail to develop eggs from the small meal also fail to develop any behavioral inhibition. Mosquitoes that oviposit during the 48 h following a blood meal return to seek a host by 72 h (Fig. 2).

The behavior outlined in Fig. 2 has several implications for multiple feeding behavior. First, not all mosquitoes that ingest small meals will

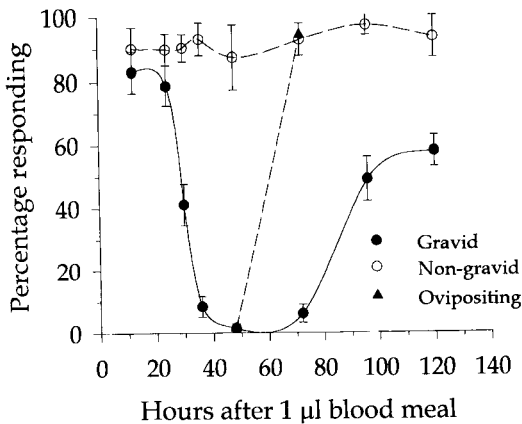


Fig. 2. Percentage of mosquitoes responding to host stimuli in an olfactometer after receiving 1- μ l blood meals. At 48 h, one group was allowed to oviposit and was tested again at 72 h.

develop eggs. There is a threshold volume required to initiate and sustain egg maturation that is dependent on overall nutritional state (Edman and Lynn 1975, Klowden 1986). If eggs fail to develop after blood ingestion, females will continue to engage in host seeking. Second, mosquitoes that do develop eggs from the small blood meals will not show any behavioral inhibition, once the distention has been reduced from digestion, until at least a day after feeding. Third, some gravid mosquitoes will return to seek a host if they are unable to lay their eggs. In the absence of oviposition, about half the gravid population of *Ae. aegypti* engage in host seeking at 96 h.

We can only speculate on the possible reasons for the evolution of mechanisms that inhibit something as important as the acquisition of a reproductive diet. Edman (Edman and Kale 1971, Edman et al. 1972, Edman et al. 1984) has demonstrated that vertebrate hosts are far from cooperative and often display defensive behavior directed against mosquito feeding attempts. As a result of this defensive behavior, an intense selective pressure may have shaped the evolution of mechanisms that prevented the female mosquito from taking the risk of approaching a host when it has already fed and the 2nd meal would be of little reproductive value. Therefore, these endogenous mechanisms allow the mosquito to minimize the risks inherent in approaching a host by restricting this risky behavior only to certain times when blood ingestion would indeed affect reproduction. It makes good biological sense to avoid a confrontation with a large host when another blood meal would not be reproductively advantageous.

Factors that modulate the inhibition of host seeking: Mosquitoes often fail to abide by the

physiological rules that regulate host-seeking behavior, evidenced by frequent reports of multiple feeding (Macdonald 1956, Muul et al. 1975, Boreham et al. 1979, Mitchell and Millian 1981). If physiological mechanisms exist to prevent mosquitoes from feeding, then how can we explain the recovery of mosquitoes from attractant traps that show the presence of mixed blood meals, and the continued host-seeking behavior of females that already have ingested blood? Given the 2 basic mechanisms that inhibit behavior after a large blood meal and during egg development, whether host-seeking behavior is indeed expressed is determined largely by the physiological state of the mosquito.

Considering the defensive behavior displayed by many of the hosts that mosquitoes feed upon (Day and Edman 1984, Edman and Scott 1987), refeeding shortly after an initial blood meal may be most likely due to interruptions while the mosquitoes were feeding, causing them to ingest initial blood volumes that are at or below the distention threshold for that individual (Klowden and Lea 1979c). A mosquito that ingests less than its distention threshold will continue to seek a host. The effect of distention and the rate it is reduced is also related to aging. Older *Ae. aegypti* have a lower distention threshold for the blood volumes that are necessary to terminate host seeking, but if these females have undergone a previous gonotrophic cycle, there is no age-related change (Klowden and Lea 1984). However, the rate at which the gonotrophically older mosquitoes return to host seeking is affected. When 20-day-old mosquitoes were given 3 μ l of blood, the females that had undergone a previous gonotrophic cycle were more likely to return to host seeking following the meal (Fig. 3). This may be due to their age-related changes in digestive physiology (Briegel 1981). Because the older segment of a mosquito population also has the greatest opportunity for acquiring parasites, age-related differences are of significant epidemiological concern.

The nutritional state of the female herself probably has the greatest effect on host-seeking behavior. Marginally nourished females are less likely to reproduce when given a blood meal, and in the absence of egg development, are less likely to show host-seeking inhibition. Even with eggs, however, the behavioral inhibition is significantly reduced when mosquitoes are not adequately nourished (Klowden 1986). The oocyte-induced inhibition is also affected by aging; gonotrophically older mosquitoes are significantly more likely to engage in host-seeking behavior while gravid (Klowden and Lea 1980).

One factor that has been very underestimated is the effect of the male mosquito on female be-

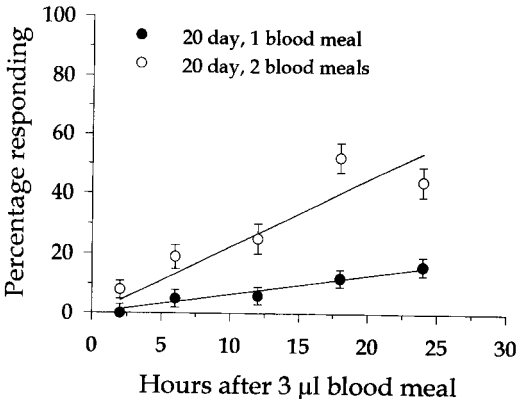


Fig. 3. Percentage of 20-day-old mosquitoes responding to host stimuli in an olfactometer after receiving 3-µl blood meals. The group represented by the open triangles underwent one previous gonotrophic cycle.

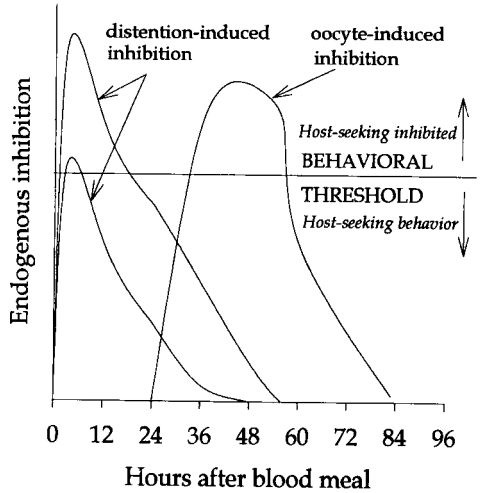


Fig. 4. Role of distention and egg development in the inhibition of host seeking. As the effect of either one increases above a behavioral threshold, host-seeking behavior is no longer expressed. Small blood meals, shown by the smaller distention curve, would be less likely to inhibit behavior because they do not significantly exceed this threshold. The level of the behavioral threshold is set by physiological state.

avior. An unseminated female is more likely to engage in host seeking while she is developing her eggs (Lavoipierre 1958, Klowden and Lea 1979a). This correlates with ecological evidence that *Ae. aegypti* males are attracted to hosts in the field (Hartberg 1971), and this reduced host-seeking inhibition in unmated gravid females may provide a mechanism whereby they can return to a host, not necessarily for a blood meal, but to increase their chances of becoming inseminated. The effect of mating appears to result specifically from the activity of male accessory gland substances that are transferred from the male. The injection of male accessory gland substances into gravid unmated *Ae. aegypti* increases the inhibition compared to saline-injected controls (Fernandez and Klowden, unpublished data).

Male diet is also significant. The protein content of accessory glands from males that were maintained only on water after emergence is reduced, and when these males mate, their contribution to the females is less effective in inhibiting host seeking during oogenesis (Fernandez and Klowden, unpublished data). Given the relatively poor nutritional state of field populations of mosquitoes (Day and Van Handel 1986), poorly nourished males mating with females in the field may not provide the quantity or quality of substances necessary to inhibit the host seeking of females during egg maturation.

Figure 4 displays a model for the control of host-seeking behavior after a blood meal. Host-seeking behavior occurs as long as information from the 2 mechanisms of distention-induced and oocyte-induced inhibition remain below the behavioral threshold. However, if the meal is large enough to exceed this threshold, it inhibits

subsequent host seeking until digestion reduces the distention to below the threshold. If eggs mature, a humoral inhibitor begins the 2nd phase of the inhibition until eggs are laid. A smaller meal and rapid digestion may be responsible for a brief period of host-seeking behavior before oocyte-induced inhibition is initiated. A larger meal may maintain the inhibition long enough so that the 2 mechanisms overlap and inhibit host seeking throughout the gonotrophic cycle. Endogenous factors such as nutritional state, age, and male accessory gland substances change the

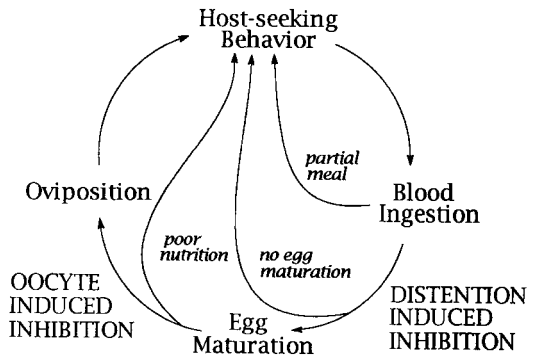


Fig. 5. Modification of the traditional gonotrophic cycle, taking into account the factors that can cause host-seeking behavior to return after an initial blood meal.

level of the behavioral threshold, shifting it up or down and modulating the effectiveness of the 2 mechanisms.

Generalizing these mechanisms of behavioral inhibition to all mosquito species may not be prudent. Our preliminary experiments with several anophelines have demonstrated that some species in this group may not have evolved the same mechanisms as aedines for inhibiting host-seeking behavior during oogenesis (Klowden and Briegel 1994). If defensive host behavior was indeed the selective pressure shaping the evolution of these mechanisms, anophelines, which feed during crepuscular periods and at night when hosts are less active, may not have encountered selective forces as intense as have day-biting *Aedes*.

CONCLUSIONS

For *Ae. aegypti*, the cyclic feeding that is characteristic of the gonotrophic cycle is regulated by the 2 mechanisms of distention-induced and oocyte-induced host-seeking inhibition, but can be modulated by the physiological state of the insect. Although this behavior may be best represented by well-fed, laboratory-reared mosquitoes, variations in nutrition, age, and mating status can affect this feeding frequency. The gonotrophic cycle clearly exists in laboratory populations, but it might be useful to acknowledge that smaller cycles operating within the larger cycle may better characterize the tendency of field populations to feed multiply (Fig. 5). Factors such as partial blood meals, and the poor nutritional state of both males and females, can increase the number of these subcycles within a single gonotrophic cycle. The existence of these subcycles, and the failure of many anophelines to show any behavioral inhibition during egg development, suggest that the traditional single blood meal taken during each gonotrophic cycle is no longer an adequate model upon which to base our conclusions regarding the feeding frequency of mosquito populations.

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