THE SENSORY PHYSIOLOGY OF HOST-SEEKING BEHAVIOR IN MOSQUITOES

M. F. Bowen

Insect Neurobiology Program, SRI International, Menlo Park, California 94025

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INTRODUCTION

Mosquitoes depend on receptors for a variety of sensory modalities, including vision, hearing, mechanoreception, and chemoreception, to transduce environmental information into biologically useful signals. In all likelihood, each modality plays a role in the complex process of identifying and locating appropriate blood-meal hosts. Analysis of blood-feeding behavior in the field, as well as in the laboratory, is a challenging endeavor, not only because blood feeding is a composite of behaviors and the sensory input is complex (3, 29, 37), but also because of the variety of feeding strategies exhibited by mosquitoes in their natural habitats (27) and the variations in internal and external states that impinge upon the expression of the behavior (48, 49). The inter- and intraspecific variations in behavioral response to a host add yet another layer of complexity to the analysis (37, 84).

Given such circumstances, the realization that the process of finding and taking a blood meal can be broken down into a series of discrete stimulus-response behaviors (29, 37) has provided a useful and, indeed, essential operational premise in the effort to understand how and when a mosquito takes a blood meal.

One such stimulus-response behavior is host-seeking. Host-seeking is distinct from other behaviors in the blood-feeding repertoire such as landing,

probing, and biting. This distinction is not merely academic: each behavior is mediated by different stimuli detected by distinct sets of receptors, and the behaviors can be uncoupled from each other experimentally (65, 66). Furthermore, in the natural sequence of events leading up to blood feeding, the female must locate a host before it can take a blood meal.

The females' tendency to engage in host-seeking changes in concert with variations in physiological state such as age, reproductive status, and diapause (48, 49, 67). Olfactory receptors in the mosquito undergo alterations in sensitivity that are correlated with these changes in host-seeking behavior (10, 17, 18). Although the mosquito uses both visual and olfactory cues to orient toward a host (3), the olfactory system plays a prominent role in modulating the response. This review summarizes our current knowledge of the receptors responsible for detecting host attractants in the female mosquito and the physiological factors that influence olfactory responsiveness and host-seeking behavior.

In light of the extensive literature on host location in mosquitoes, this review does not attempt to be comprehensive. Emphasis is placed on recent literature that relates to mechanisms of olfactory-mediated host attraction. Further information and earlier literature can be found in more general reviews of blood-feeding and host location (27, 29, 37, 63, 90, 91, 94) and in those covering the endocrinology of host-seeking in mosquitoes (48, 49).

BEHAVIORAL ASPECTS OF HOST SEEKING

Host-seeking has been operationally defined as the in-flight orientation of the avid female toward a potential blood-meal host. The term *host-seeking* may be too general and teleological a term (45) to be of much use in the description of neurophysiological correlates of mosquito host attraction. This definition would benefit from more precise descriptions of behavioral responses of mosquitoes under different odor and temperature conditions, clarification of the range of effectiveness of various host attractants, and knowledge of the identity and characteristics of the receptors that detect odors associated with the various behavioral responses. A meaningful analysis of odor-mediated host-seeking behavior requires knowledge of (*a*) the specific chemical components of host odors and the amount released by the odor source per unit time, (*b*) the configuration of the stimulus in time and space, and (*c*) the contribution of each host odor to the individual responses that make up the composite behavior of host-seeking.

The Chemical Identity and Concentration of Behaviorally Relevant Host Volatiles

CO_2 and lactic acid are the best-described host emanations in terms of chemical identity and amounts released into the host airstream (1, 32, 85).

Not surprisingly these attractants have received the most attention in terms of sensory physiology and behavior. This emphasis does not imply that lactic acid and CO_2 are the only host attractants for mosquitoes because the intact host is still the most effective stimulus in eliciting host-seeking behavior (32, 85), and unidentified chemicals in addition to lactic acid are attractive to mosquitoes (2, 83, 85). 1-Octen-3-ol, for example, was first identified as a host attractant for tsetse (36) but has recently been shown to attract certain species of mosquitoes in the field (46, 92). Current techniques for the capture and chemical analysis of gas-phase emanations such as gas chromatography and atomic mass spectrophotometry should unravel the chemical identity and vapor-phase concentrations of additional host attractants. A knowledge of the emitted levels of identified volatiles is essential because different concentrations of olfactory stimulants may have very different effects on mosquito behavior (32, 85).

Lactic acid has been shown to elicit oriented flight behavior in mosquitoes under laboratory conditions (1, 85). L-lactic acid is more attractive than the D-form under certain conditions, although this observation has not been reconciled with the observation that D- and L-lactic acid are equally effective in eliciting an electrophysiological response from the lactic acid–excited cell (20). Lactic acid is reportedly repellent at high source concentrations (reviewed in 85). However, the low vapor pressure of lactic acid at room or skin temperature limits the amount of the chemical in the vapor phase, so repellent effects may result from the complex chemistry of lactic acid and the presence of secondary compounds rather than lactic acid per se (53). The stimulus measurement relevant to host-seeking behavior is the concentration of the chemical in the vapor phase emanating from the host. For lactic acid, this can be calculated from the evaporation rate estimated from 11 human subjects by Smith et al (85) as a lactic acid flux rate of $15.0 \times 10^{-11} \pm 4.3$ mol/s (range $= 9.2 \times 10^{-11}$ to 24.8×10^{-11} mol/s), which is well within the stimulus dynamic range of the olfactory receptors (see section on sensory aspects of host-seeking).

Tests of lactic acid as an attractant in the field have not been successful (89), although recent field trials suggest that the compound is somewhat attractive for certain species (46). The ability (or inability) of lactic acid to elicit host-seeking behavior must be evaluated in light of two considerations: (*a*) the probability that a "multi-component chemical stimulus" (32) is required, possibly involving as yet unidentified host odors as well as CO_2 (32, 46, 85, 92); and (*b*) the effect of the physiological state of the female mosquito on odor reception (10, 17, 18).

CO_2 is an established host attractant for mosquitoes both in the laboratory and in the field (32). Gillies (32) thoroughly reviewed the effect of CO_2 on mosquito behavior. He concludes that CO_2 both activates (induces take-off and sustained flight) and orients mosquitoes. Gillies (32) further points out

that CO_2 elicits orientation in mosquitoes under laboratory conditions only when presented intermittently and that, in the field, CO_2 is probably experienced by the mosquito in this way because an odor released from a point source exists as a filamentous plume rather than a broad, homogeneous concentration gradient (70). Since both activation and orientation are part of the process of attraction, the attractive effects of other odors on host-seeking behavior cannot always be elicited unless CO_2 and the host odor are presented simultaneously. Lactic acid, for example, is ineffective in stimulating host-seeking unless CO_2 is also present (46, 85).

The concentration of CO_2 in atmospheric air is 0.03–0.04% and in human breath 4.5%. Excretion from the total human skin surface is about 0.3 to 1.5% of that expired from the lungs (see 32). Local atmospheric levels can vary considerably, depending on time of day and density of vegetation, so the CO_2 differential between atmospheric levels and biologically relevant objectives is considerable (32). Mosquitoes are electrophysiologically sensitive to changes in CO_2 levels as low as 0.01% (43). Unnaturally high CO_2 levels can have anomalous effects on behavior and physiology (71). Because CO_2 induces and maintains flight, mosquitoes may be reluctant to terminate flight and land under such conditions, particularly in the absence of other odors (32).

The Configuration of the Stimulus in Time and Space

Odor released from a point source exists in time and space as a discrete yet discontinuous plume carried downwind, usually in a turbulent airflow that frequently changes direction (70). Because of the filamentous nature and irregular path of the plume, odor is experienced downwind as a series of intermittent pulses (6). Insect olfactory receptors are well equipped to respond to such stimulation. For example, moth olfactory receptors can temporally reflect rapid, intermittent odor pulses (42, 80) and mosquitoes have fast on-off responses to lactic acid and CO_2 (23, 43). This phenomenon is also reflected at more central processing levels (e.g. 14). As demonstrated in moths and mosquitoes, the continuous presence of an odor stimulus can elicit anomalous behavioral responses that may not be representative of the response to a physiological odor source (4, 5, 32). For example, mosquitoes will not display sustained upwind flight in a wind tunnel unless CO_2 is presented in pulses (32, 74). Furthermore, the continuous presentation of stimulus can lead to adaptation in some olfactory receptors (5, 42).

Orientation and Approach to the Host

Both field and laboratory observations employing various devices such as traps, wind tunnels, and olfactometers have yielded a plethora of data regard-

ing the role of odors in mediating host-seeking behavior (29, 37). The data are difficult to compare, perhaps because of variations in (a) species involved, (b) physiological state of the experimental subjects, (c) techniques employed, and (d) conditions under which the experiments were carried out. The most informative techniques, from the standpoint of the neurophysiological correlates of behavior, involve the visual analysis of flight behavior of individual insects under various odor conditions either in a wind tunnel or in the field (e.g. 15, 30). The videotaped data can be readily analyzed according to the protocol of Marsh et al (54). Flight characteristics such as duration, net ground velocity (distance along wind line per unit time), number of turns, turning frequency, turning severity (degrees per turn), angular velocity, and interreversal span (distance between reversal points measured perpendicular to the midline of the plume) can all be obtained from the video recordings.

Such studies are not yet widely available for mosquitoes but have been immensely informative in the analysis of moth pheromone orientation (4, 5). When pheromone is detected, male moths fly upwind, using visual cues to control speed and direction (optomotor anemotaxis), and initiate an internal program of self-steered counter-turning. The behavior is thus a product of both an internal program (idiothetic control) and external stimuli (allothetic control).

Researchers have good reasons to suspect that mosquitoes orient to hosts in a way similar to moths because most mosquitoes fly upwind to the source of host odors (see 92). The pattern and mechanism of upwind orientation in the mosquito may not be exactly identical to that in the Lepidoptera because the mosquito-host relationship is one of predator and prey, whereas mate-finding in the Lepidoptera is an intraspecific interaction of mutual advantage to both odor-emitter and recipient. For example, the hematophagous tsetse does not use self-steered counter-turning, although it does employ optomotor anemotaxis (15). Preliminary studies in our laboratory suggest that the mosquito also employs an irregular flight pattern similar to that seen in the tsetse. Any adaptive advantage to a regular versus an irregular flight path in predatory insects is yet to be determined. Also, the issue of how mosquitoes can orient upwind to odor sources in the dark remains unresolved. Mosquitoes can use visual cues in upwind orientation, as first demonstrated by Kennedy (44) in the *Aedes aegypti*. Gillett (31) has suggested that mosquitoes do not need visual cues to fly upwind but can do so by flying close to the ground and making periodic dips to detect wind shear. In addition, species are different with respect to the details of upwind flight behavior and the conditions under which it can be elicited (26, 46, 74, 89, 92). More studies on flight patterns of mosquitoes in response to host volatiles, both in the laboratory and in the field, are needed.

SENSORY ASPECTS OF HOST-SEEKING

Morphology and Ultrastructure of Olfactory Sensilla

Numerous studies have focused on the structure of the antennal sensilla in mosquitoes (39, 55–59, 61, 62). Similar morphology has been found in culicine and anopheline mosquitoes (39, 55, 56, 58, 61). Sensilla are cuticular extensions that house the sensory cell dendrites. The cell body is located at the sensillar base; the dendrites reside within the cuticular extension; and the axonal afferent nerves from many different sensilla are bundled together into two flagellar nerves that extend proximally before eventually synapsing with interneurons in the antennal lobes of the deutocerebrum (12, 38). In *A. aegypti,* for example, eight morphological types can be distinguished (58); (*a*) sensilla chaetica containing one neuron (mechanoreception), (*b*) sensilla ampullacea containing three neurons (probably thermoreception), (*c*) sensilla coeloconica or pit pegs containing three neurons (temperature reception), (*d*) sensilla basiconica or grooved pegs containing three to five neurons (olfaction), and (*e*) four types of sensilla trichodea (long pointed, short pointed, blunt-tipped type I and blunt-tipped type II), all of which house one or two olfactory neurons. The sensillar types are not uniformly distributed over the antenna and the total number of each type varies from six sensilla coeloconica per antenna to 507 blunt-tipped type I sensilla trichodea per antenna (58). Of a total of approximately 2,058 neurons per flagellar nerve, 93% are associated with known olfactory receptors (58). The thin-walled bulb-shaped organs, or pegs, on the palps each contain three neurons (56), at least one of which is sensitive to changes in CO_2 (43).

Electrophysiology

Ultrastructural studies have been necessary to identify specific sensilla and to determine the number of neurons associated with each. Electrophysiological studies of a single sensillum provide positive functional identification of the olfactory neurons housed within. Detailed, quantitative studies of olfactory electrophysiology of host attractants in mosquitoes have been limited to *A. aegypti* and *Culex pipiens.*

Single-unit extracellular recordings have identified three types of neurons that detect host-derived stimuli: lactic acid, CO_2, and temperature. Each is associated with a morphologically distinct sensillar type. The response characteristics of these sensory receptors are typical of receptor cells in many other organisms (76).

LACTIC ACID–SENSITIVE CELLS   The sensilla basiconica or grooved pegs (57) contain receptors that are sensitive to lactic acid (23). One type responds to lactic acid with an increase in spike frequency and a second type exhibits a

decrease in spike frequency. The dynamic range (range of stimulus intensities over which a receptor or population of receptors can respond without saturation) is the same for both the excited and inhibited cells. Individual cells display variable dynamic ranges (range fractionation) within an effective total range of between 2.7 and 40.0 $\times$ 10^{-11} mol/s (20). Lactic acid flux from a human hand falls well within this range (21, 85). In contrast to Kellogg (43), Davis & Sokolove (23) found that humidity responses were of insufficient sensitivity for the cells to be considered humidity receptors, i.e. 20 impulses/s for a 50% change in humidity; the cells merely depended on humidity for proper functioning (see also 86).

The grooved peg neurons are unresponsive to chemicals other than those closely related to lactic acid. The optimal stimulus configuration for these cells is a 3-carbon, α-hydroxy, monocarboxylic acid. Although the requirement of the α-side group is not rigidly specific (20), the cells display the highest sensitivity to lactic acid. The receptor does not discriminate between the l and d isomers of lactic acid (20).

The sensilla basiconica usually contain three but may have as many as five neurons (57) so that receptors sensitive to chemicals other than lactic acid may also be contained within the grooved pegs.

CO2-SENSITIVE CELLS   The behavioral synergism between CO_2 and other host odors must occur centrally rather than at the peripheral level because most known odor receptors reside on the antennae whereas the receptors for CO_2 are located on the palps (43). The club-shaped pegs each house three neurons, one of which detects changes in CO_2 (43). The cells exhibit phasic-tonic responses to fluctuations in CO_2 and logarithmic sensitivity to stimulus. Changes in CO_2 levels as low as 0.01% can be detected. The receptors are apparently saturated at 4.0% CO_2, the level present in human breath. Although another cell found in this sensillum responds to organic solvents such as acetone, n-heptane, and amyl acetate, the published data do not indicate whether this response was physiological or pharmacological because the stimulus intensities were not given (43). The three neurons innervating each peg consist of two morphologically distinct types of cells (56). Which cell type is CO_2-sensitive and the quantitative response characteristics of the other neuron are unknown.

THERMORECEPTORS   Mosquitoes have acutely sensitive thermoreceptors located in the sensilla coeloconica (22). These consist of two types of cells: one displays an increase in spike frequency in response to sudden increases in temperature; the other increases its firing rate in response to decreases in temperature (22). The spontaneous firing rates of the cells depend on ambient temperature and the changes in spike frequency observed upon a step change

in temperature depend on the starting temperature. The maximum response is observed at an ambient temperature of between 25° and 28°C. Maximum phasic sensitivity is observed in response to temperature changes of ± 0.2°C, but the cells can respond to changes as low as 0.05°C. Warm, moist convection currents arising from a host are important host-seeking cues, and currents having local thermal differentials of as much as 0.05°C exist at distances greater than two meters away from a 2- to 3-kg rabbit (E. E. Davis, unpublished observations). Such temperature changes are well within the range of detection of the mosquito thermoreceptors.

Neural Coding Characteristics

With respect to the mechanisms for encoding a specific behavior pattern, the terms *odor generalist* and *odor specialist* refer to the degree of specificity of a receptor for a given stimulus (42). The terms *labelled line* and *across fiber pattern* refer to the interpretation (i.e. perception) of the sensory signal by the central nervous system and its behavioral consequence (42). The characteristics of the mosquito olfactory receptors that have been examined so far indicate that these neurons are odor specialists, i.e. they respond to a relatively narrow range of chemical stimuli. Input from one type of odor specialist, CO_2, is adequate for orientation. In this context, the CO_2 receptor could be considered a labelled line. The input from several such odor specialists (receptors for lactic acid, CO_2, and temperature, and, possibly, receptors for as-yet-unidentified host odors) is necessary to evoke the complete response leading to the location and identification of an intact host, i.e. an across fiber pattern of odor specialist cells.

In the case of the lactic acid–sensitive cells, receptor sensitivity has been shown to be directly related to host-seeking behavior. The tendency of a given population of females to exhibit host-seeking behavior changes in conjunction with physiological events such as vitellogenesis and diapause (see section on sensory and endocrinological aspects of host-seeking). Actively host-seeking females invariably have receptors that are highly sensitive to lactic acid, but only low-sensitivity receptors are found on non-host-responsive females (10, 17, 18). When host-seeking behavior is inhibited, the dynamic range of the lactic acid–excited cells shifts to stimulus intensities that exceed the expected maximum lactic acid emission from a human hand (21). The response to stimulus of the lactic acid–excited cell is inhibited by the repellent DEET (N,N-diethyl-m-toluamide) (19). The lactic acid–inhibited cell does not undergo such changes in sensitivity (21).

Based on the net response-firing patterns of both types of neurons in host-responsive and non-host-responsive females in response to lactic acid alone and in combination with DEET, a model of the sensory control of host-seeking behavior has been proposed that considers the response charac-

teristics of both the lactic acid–excited and –inhibited cells (21). The model
assumes that host-seeking behavior is directly related to the total activity in
this set of neurons. The role of the inhibited cell is to actively enforce
host-seeking inhibition by decreasing the net afferent output to below the
spontaneous firing rate when the sensitivity of the lactic acid--excited cells
shifts to a lower sensitivity state (21). Such a net decrease is observed during
egg maturation (see 18), during diapause (10), and in the presence of the
repellent DEET (19), and each situation is characterized by the absence of
host-seeking.

SENSORY AND ENDOCRINOLOGICAL ASPECTS OF HOST-SEEKING

Young and Nulliparous Females

Most female mosquitoes do not become host-responsive until several days
after pupal-adult emergence (see 18). The appearance of host-seeking be-
havior in A. aegypti coincides with a progression in lactic acid–receptor
sensitivity, which suggests a developmental process. Newly emerged (0–24 h
post-emergence) females are nonresponsive and possess only silent (nonspik-
ing) neurons. Older females (24–96 h post-emergence) that are not host-
responsive possess more spiking neurons, most of which are nonresponsive or
nonspecific; some neurons show specificity for lactic acid but have low
sensitivity. Females of any age that exhibit host-seeking behavior (18–24
hours post emergence and older) possess neurons that are highly sensitive to
lactic acid (18). The appearance of this behavior in A. aegypti roughly
coincides with juvenile hormone-dependent previtellogenic ovarian develop-
ment, but the correlation is incidental: juvenile hormone deprivation by
allatectomy (removal of the corpora allata) within one hour of adult emer-
gence fails to prevent the appearance of host-seeking even though ovarian
follicles remain teneral (9). Lactic acid sensitivity is likewise unaffected by
allatectomy (9). In A. aegypti, host-seeking behavior must be either in-
dependent of juvenile hormone or have a sensitivity threshold and/or sensitive
period very different from that of the ovaries (28).

In C. pipiens, the development of host-seeking behavior after emergence
may be juvenile hormone dependent. Movement toward a host is not observed
in females that have been allatectomized at one hour post-emergence (R.
Meola & J. Readio, personal communication). This effect was assessed using
paired cages, of which one contained a host and the other contained ex-
perimental or control groups of mosquitoes. Only 1 of 92 allatectomized
females moved to the host cage in overnight trials. Allatectomized females in
which the corpora allata had been re-implanted moved readily to the host cage
in overnight trials, and 5, 10, and 50 ng of the juvenile hormone analogue

methoprene also restored the behavior in a dose-dependent manner. The movement of unoperated females in the absence of a host was similar to that of allatectomized females in the presence of a host. The effect of juvenile hormone-deprivation on host attractant-receptor sensitivity in *Culex* has not been explored. The finding that allatectomy affects receptor sensitivity would also support the notion that juvenile hormone initiates host-seeking behavior in *Culex;* however, a lack of a juvenile hormone–deprivation effect on receptor functioning would not preclude a role for juvenile hormone in host-seeking behavior. The behavior can also be modulated at levels other than the periphery, as during distension inhibition (see section on blood-fed and gravid females) and during the circadian cycle (see section on the circadian system).

Blood-Fed and Gravid Females

The immediate effect of a blood meal above a threshold volume (2.5 μl in young *A. aegypti* females) is inhibition of host-seeking resulting from the activation of stretch receptors that reside in the anterior part of the abdomen (reviewed in 48, 49). Whether this distension inhibition is mediated directly by nervous signals or by hormonal intermediaries is unknown. Whatever the mechanism of distension inhibition in mosquitoes, the inhibition is not effected peripherally: olfactory receptor sensitivity is unaffected by distension; lactic acid receptor sensitivity remains high for about 18–24 h after the blood meal (17).

After the blood meal is digested and distension is alleviated, humoral events related to oocyte maturation in mated females inhibit host-seeking until after oviposition, a phenomenon referred to as *oocyte-induced behavioral inhibition* (reviewed in 48, 49). In blood-fed females that go on to develop eggs, the ovaries release an initiating factor 6–12 hours after blood feeding. The ovarian factor stimulates the fat body to produce a hemolymph-borne substance that either directly or indirectly renders the peripheral olfactory receptors less sensitive to lactic acid (51).

The identities of the ovarian and fat body factors are unknown, although some evidence suggests that the ovarian initiating factor released within 12 h after a blood meal is an ecdysteroid (11). Large doses of ecdysteroids can inhibit host-seeking (7), but this effect is pharmacological and nonspecific (47).

The mechanism by which olfactory receptor sensitivity is shifted is unknown. The morphological arrangement of insect receptor neurons within the sensillum suggests several routes by which receptor function could be controlled via hemolymph-borne signals (8). The cell body lies at the base of the sensillum, which is filled with extrahemolymphatic fluid called receptor lymph. The dendrites reside within this lymphatic compartment, which has a

composition apparently related to receptor function: high K^+ concentration (24) and stimulus-binding and degradative proteins (95). The receptor lymph and the receptor cells themselves are exposed to hemolymphatic signals such as hormones, and alterations in either could result in changes in receptor functioning (8). Although direct humoral control of receptor function has not been demonstrated, anatomical (60, 75), electrophysiological (8, 17, 51), and behavioral (8, 17, 51) experiments all suggest that such control can and does occur. The possibility that the antennal receptors are subject to efferent neural control cannot be ruled out, although to date such innervation has not been demonstrated.

HOST-SEEKING IN MOSQUITOES THAT UNDERGO ADULT DIAPAUSE

Many temperate-zone mosquitoes overwinter in a state of dormancy called diapause, during which reproduction and development are suspended. Diapause is induced by endocrine changes implemented through the reception of seasonal photoperiodic and thermal cues that presage the onset of inimical climatic conditions. The occurrence and physiology of embryonic, larval, and adult diapause in mosquitoes are the topics of a recent and comprehensive review by Mitchell (67), and adult diapause in *Culex* mosquitoes has also been recently reviewed (28).

The effect of diapause on host-seeking behavior has been examined only in species that undergo adult reproductive diapause. Diapausing females (adult male mosquitoes do not survive the winter) are inseminated, possess teneral ovarian follicles, and have greatly hypertrophied fat bodies resulting from increased lipid deposition (28, 67, 96). Depending on species and strain, diapausing females can show one of two patterns of blood-feeding behavior during diapause (reviewed in 96). Some groups may take occasional blood meals during diapause without developing eggs, a phenomenon known as *gonotrophic dissociation*. Other mosquitoes do not blood-feed or develop eggs during diapause, a situation referred to as *gonotrophic concordance*. Concordant mosquitoes depend entirely on plant juices to build up the lipid reserves necessary for successful overwintering (67, 96).

The best-described case of gonotrophic concordance is that of *C. pipiens*. This species does not host-seek during diapause (10, 66) but can be induced to bite and take a blood meal if placed in close proximity to a host (66). When blood feeding is induced in diapausing females, the meals taken are sub-threshold or incompletely digested and prematurely excreted, and thus not used for fat body development (68, 69). Analysis of the peripheral receptors corroborates these observations. The lactic acid–sensitive cells in diapausing females consist of low-sensitivity, nonspecific, and nonresponsive neurons

whereas high-sensitivity receptors are present on both nondiapausing and postdiapausing females (10). Highly sensitive receptors are not found on diapausing animals (10), a condition that is reminiscent of that in teneral and gravid *A. aegypti* females (17, 18). The question arises, then, whether the diapause condition represents an interrupted state of imaginal development (akin to that in teneral *A. aegypti*) or whether the sensory system is mature, albeit inhibited (as in the gravid *A. aegypti*). Nonresponsive, nonspecific, and silent neurons may reflect undifferentiated receptors, so estimation of their numbers can give some idea of the stage of development of the nervous system. Preliminary data, showing the presence of primarily undifferentiated neurons in diapausing females, suggest that the peripheral sensory system in diapausing females is in a state of interrupted or delayed development (M. F. Bowen, in preparation). Although the data for the lactic acid–sensitive cells are inconclusive, the analysis of other receptor-cell groups (specifically, the neurons located in the sensilla trichodea that are sensitive to oviposition site-related volatiles) suggests that diapause interrupts peripheral sensory development: the number of nonresponsive neurons is higher in diapausing females than in nondiapausing or gravid females. These observations suggest that the peripheral sensory system undergoes developmental processes in early imaginal life and that this development can be influenced by physiological state so as to affect adult behavior.

Gonotrophic dissociation was first demonstrated in *Anopheles labranchiae atroparvus*, a species that remains sequestered in domestic shelters during the fall and winter and continues to take blood meals during this time without developing eggs (see 96). The phenomenon has since been confirmed in other anophelines (96). As Washino (96) points out, gonotrophic dissociation may occur only intermittently throughout the geographic range of a given species and the incidence of blood-feeding can vary considerably between populations. Because fat body development was roughly equivalent in dissociative and concordant *Anopheles freeborni* females and the survival rates of the two types of diapausing females did not differ, Washino (96) concluded that the selective advantage of blood-feeding during diapause was related to the relative availability of blood-meal hosts as compared to plant nutrient sources.

With respect to host-seeking behavior and its relationship to gonotrophic dissociation, several points require clarification. 1. It is not clear if females that exhibit gonotrophic dissociation can host-seek strictly speaking. 2. If host-seeking is indeed expressed by such females, the fact that this behavior can persist independently of physiological changes attendant on the diapause state suggests that the behavior in anophelines, unlike that in *Culex,* is not strictly controlled by diapause-inductive processes but is somewhat labile. The physiological conditions that result in the expression or nonexpression of

blood-feeding behavior in these diapausing mosquitoes are unknown. 3. The sensory physiology of dissociation females has not been examined so one cannot evaluate the role of the peripheral sensory system in gonotrophic dissociation at this time.

Washino's (96) statement that "the physiological mechanism of diapause expressed as gonotrophic concordance or dissociation still is unclear and requires considerable clarification" remains relevant. The endocrinology of adult diapause in mosquitoes is not well understood. The failure of the follicles to develop is generally believed to result from low juvenile hormone titers in the adult (reviewed in 28, 67). Mitchell (67) points out that because the corpora allata do not affect lipid and glycogen synthesis, the involvement in diapause of the medial brain neurosecretory cells is a distinct possibility because these cells have been shown to control this metabolic pathway in other species. The analysis of diapause in mosquitoes, as well as in other insects, is blocked by a lack of basic information on hormone titers during development and complicated by the fact that the environmental cues that induce diapause are perceived in stages prior to that in which diapause is expressed. In adult diapause in mosquitoes, the larva and pupa are the stages sensitive to diapause-inducing stimuli (reviewed in 67). Although covert, the juvenile-stage events that result in adult diapause should be amenable to analysis using current techniques. The internal and external larval milieu may have profound effects on adult behavior as well as sensillar morphology and receptor functioning (see below). The effect of rearing conditions on adult behavior in nondiapausing as well as diapausing mosquitoes is largely un-explored and deserves more attention.

OTHER PHYSIOLOGICAL FACTORS IMPINGING ON THE EXPRESSION OF HOST-SEEKING

Nutritional State

Both larval and adult nutrition can affect the expression of host-seeking behavior in adult females. Rearing A. aegypti larvae on a suboptimal diet gives rise to adults that are not only smaller in size but also less likely to engage in host-seeking behavior (50). Providing sugar to such adults fails to increase host responsiveness (50). Because Culex larvae reared on a sub-optimal diet can metamorphose into adults with impaired flight capacity (16), the ability to fly, rather than host-seeking per se, may be affected by larval nutritional conditions in A. aegypti.

Sugar-feeding in the adult can affect host responsiveness immediately after sugar ingestion (40) as well as during vitellogenesis: Sugar-deprived A. aegypti females are more likely to exhibit host-seeking behavior after a blood meal regardless of whether they develop eggs (reviewed in 48, 49). Such

increased host-seeking is believed to result from the absence of oocyte-induced inhibition (reviewed in 48, 49). One would expect the peripheral host-attractant receptors to display high sensitivity in sugar-deprived, host-responsive females.

Aging

Aging alters host-seeking behavior in several ways; the effects depend upon whether the female is chronologically or gonotrophically old (reviewed in 48, 49). First, the threshold blood volume for distension inhibition is lower in chronologically old females. Second, the recovery of host-seeking after distension inhibition occurs more rapidly in gonotrophically aged females than in chronologically aged females, possibly because of faster blood digestion. Third, the onset of oocyte-induced inhibition of host-seeking behavior is delayed and the incidence is diminished as both gonotrophic and physiologic age increase.

The effect of aging on peripheral receptor sensitivity in mosquitoes has not been systematically examined. Age-related changes in receptor function occur in other Diptera. In blowflies, for example, the numbers of responsive salt and sugar receptors as well as the sensitivity of the remaining operative cells decrease with age in blowflies (77, 87, 88). The effect of chronologic as well as gonotrophic age on olfactory receptor functioning in mosquitoes deserves more attention, particularly since older populations in the field are of considerable epidemiological significance (48).

The Circadian System

Mosquitoes exhibit daily periods of activity and inactivity that are the external manifestations of endogenous circadian oscillators (41). Spontaneous flight activity has received the most attention, but host-seeking behavior is also expressed in a circadian pattern in many species (64, 78, 93). To express host-seeking behavior, the mosquito must be willing to fly, but evidence indicates that the two behaviors are not tightly coupled temporally and peaks of flight activity can precede and/or lag behind host-seeking (64, 79). Host-seeking behavior in *Culex* is expressed during the dark phase in both the laboratory (10) and in the field (64). The sensitivity of the lactic acid receptors does not vary throughout the day (M. F. Bowen, in preparation). High sensitivity to lactic acid can be observed in the light phase (when females are not host-responsive) as well as in the dark phase (when females are host-responsive) of the light-dark cycle. Thus, the control of the daily expression of host-seeking behavior in this species does not reside at the level of the peripheral sensory receptors.

Morphological Variation

Interspecific variations in sensillar number and density in mosquitoes have
been described (55, 59, 61, 62). The significance of these variations is not
clear, but the evolution of autogeny in the Culicidae offers one possible
explanation (59). *Wyeomyia*, for example, is a genus that has both autogenous
and anautogenous representatives. This group has retained piercing mouth-
parts, so differences in mouthpart morphology must not cause the loss of the
blood-feeding habit in autogenous members of this group (25). The density of
grooved peg sensilla in an autogenous, non-blood-feeding strain of *Wyeomyia
smithii* is significantly lower than that in the anautogenous *Wyeomyia apor-
onema* (62). If the grooved pegs in this genus house host-attractant sensitive
receptors, as in other mosquitoes, then the loss of sufficient afferent input
from this set of sensory cells might account for the absence of blood-feeding
behavior. This hypothesis would be greatly strengthened if intraspecific
differences in sensillar number between blood-feeding and non-blood-feeding
strains could be demonstrated.

In mosquitoes, autogeny is associated with precocious sexual receptivity
and advanced ovarian maturation. Both of these phenomena are juvenile
hormone dependent (33–35, 52), and studies in roaches suggest that this
morphogenic hormone can also affect sensillar number. Elevated or pro-
longed preimaginal juvenile hormone levels reduce sensillar numbers and
affect response to pheromone in adult roaches (81, 82). Experimental evi-
dence from a non-blood-feeding strain of *W. smithii* that is autogenous for
successive gonotrophic cycles (73) suggest that an early release of juvenile
hormone in the pupal stage is responsible for precocious sexual maturation
(72). One can speculate that juvenile hormone may also reduce sensillar
numbers, thus limiting the number of receptors available for the detection of
host attractants and rendering the strain less inclined to engage in host-
seeking. This hypothesis remains to be tested.

OVERVIEW AND PROSPECTS FOR FUTURE RESEARCH

It has become apparent in recent years that the mosquito sensory system is not
merely a passive conduit of information from the environment to the central
nervous system. Besides the classic sensory functions of transduction and
high-gain amplification of specific external chemical signals, olfactory
neurons apparently contribute to the control of behavioral output by modulat-
ing sensory input. This rather surprising phenomenon comes about through a
combination of the specific neural coding characteristics of the peripheral
olfactory neurons and their responsiveness to systemic signals that act to
change receptor sensitivity.

Sensory physiology does not provide a complete answer to the question of

how behavior is controlled in mosquitoes, but, as Hocking (37) pointed out, "behavioral work is always more difficult in the absence of an adequate knowledge of sensory physiology." Our current understanding of mosquito behavior would greatly benefit from comparative studies of species of mosquitoes other than *Culex* and *Aedes*. Identification of the endogenous factors that act on peripheral receptor sensitivity would facilitate the study of the mechanism of receptor sensitivity modulation, not only by endogenous signals, but also by exogenous factors such as repellents. The effects of the larval environment, senescence, learning, and other host attractants on host-seeking and receptor functioning need to be incorporated into current models of host-seeking behavior. The mosquito has been treated much like a black box in the input-output analysis of behavior, and knowledge of central processing and projection patterns of sensory afferents would greatly expand our comprehension of mosquito host-seeking as it has for behavior in other taxa (13, 14, 38). Finally, more functional terminology that describes what the mosquito "is actually doing" rather than its "presumed state of mind" (45) would greatly facilitate the analysis of host attraction in mosquitoes.

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