*Journal of the American Mosquito Control Association,* 10(2):316–325, 1994


# SENSORY PHYSIOLOGICAL BASIS FOR ATTRACTION IN MOSQUITOES

E. E. DAVIS AND M. F. BOWEN

*SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025*

ABSTRACT. Hematophagous insects use air-borne chemical cues to guide them to resources such as blood-meal hosts, plants, and oviposition sites. Research that combines behavioral and electrophysiological approaches to the study of how insects find these resources can result in useful information about what chemical signals a mosquito can detect and at what airborne concentrations such compounds are effective. Such studies have helped clarify the role of lactic acid, ammonia, carbon dioxide, octenol, phenols, temperature, and humidity in the attraction of mosquitoes, tsetse flies, and ticks to blood-meal hosts. Egg raft pheromone, indoles, cresols, methyl cyclohexanol, 2-butoxy ethanol, and fatty acid esters have been examined with respect to oviposition site location and selection. Plant volatiles have received less attention but electrophysiological responses to terpenes and green plant volatiles have been observed. Information from studies of this type can be useful in the design of both attractants and more effective repellents.

## INTRODUCTION

Mosquitoes and other arthropod disease vectors locate distant resources such as blood-meal hosts, plant resources, and oviposition sites by using chemical and physical signals that are detected by sensory receptors on the antennae. Insects can respond to the same environmental signals in different ways, depending upon their genetic background, expressed phenotype, and physiological state. Aside from interspecific differences in response to chemical signals (see reviews by Takken 1991, Kline 1994), the expression of such genetically determined developmental strategies as anautogeny, autogeny, and diapause (which in certain instances can be modified by environmental conditions) is characterized by behavioral adaptations in the insect's responsiveness to environmental signals. For example, nulliparous anautogenous mosquitoes readily respond to the odors of a blood-meal host, whereas autogenous and diapausing mosquitoes do not respond (Bowen 1990, 1991, 1992b). Once a blood meal has been taken and egg development has been initiated, however, physiological changes in anautogenous females render them unresponsive to host odors (Klowden and Lea 1979b). Some differences in responsiveness to biologically relevant signals are mediated by changes in the sensitivities of peripheral receptor neurons, and others are due to changes occurring more centrally in the insect's nervous system. For example, mosquitoes that have recently ingested a blood meal of a critical volume fail to respond to host odors because of distention inhibition, a central nervous system phenomenon (Klowden and Lea 1979a). As the eggs develop, a humoral signal down-regulates peripheral receptor sensitivity and thus renders the mosquito less sensitive to host attractants (Davis 1984a). Likewise, diapausing female mosquitoes possess host attractant receptors of low sensitivity (Bowen et al. 1988) but diapause termination is accompanied by the appearance of host-seeking behavior and high receptor sensitivity (Bowen 1990).

The research in our laboratory is directed toward understanding the role of the peripheral receptor systems in the control and regulation of odor-mediated behaviors in disease-transmitting organisms. Our approach is an integrated one. First, we determine the specific behavioral responsiveness of an individual mosquito. Then, using electrophysiological techniques, we determine the response characteristics of its peripheral receptors to environmental signals that mediate the behavior in question. Finally we confirm the physiological state of the mosquito by subsequent microdissection and examination. To obtain mosquitoes in a desired physiological condition prior to the foregoing process, we may also manipulate the physiological state of the insect by controlling access to diets, mates, and other factors and/or by microsurgically manipulating neurosecretory and other tissues and fluids, such as the corpora allata, ovaries, fat body, male accessory glands, and the mosquito's own hemolymph. In this manner, we can make precise correlations between an individual mosquito's behavior, sensory activity, and physiological state.

This presentation reviews what we know about the sensory receptors that provide the mosquito with the information it needs to detect, orient to, and arrive at a resource over a distance and to begin to sample the resource. In this review we will relate the putative sensory structures described by Sutcliffe (1994) with the series of earlier presentations describing various environ-

mental signals that affect the behavior of mosquitoes and other vector organisms.

## METHODS FOR STUDYING THE ELECTROPHYSIOLOGICAL RESPONSIVENESS OF THE SENSORY ORGANS

*Electrophysiological recording system:* Standard techniques are used to record the extracellular electrophysiological activity of the mosquito's sensory neurons. Briefly, an uninsulated tungsten microelectrode (< 1 μm tip diam) is inserted into the hemolymph space at the tip of the antenna (reference electrode) with the aid of micromanipulators at a magnification of about 900×. A similar microelectrode is inserted through the cuticle at the base of a sensillum (recording electrode) and connected to various electronic devices to amplify and record the action potentials (spikes) of the sensory neurons within the sensillum. Because there is almost always more than one sensory neuron within the sensillum and they rarely have identical response properties, it is necessary to separate their activities. Discrimination between the different sensory neurons based upon their respective amplitudes, when such differences are observed, is one means, albeit tedious and imperfect. Recently, software has become available for personal computers to sort spikes from multiple neurons into categories based upon the number of neurons present and selected temporal and amplitude parameters of the action potential shapes, the result being a set of templates to which each spike is then compared and classified (Smith et al. 1990). Stimulus intensity–response functions can be generated for each class of sensory neuron.

We record the responses of sensory neurons within single sensilla because this method allows us to identify and characterize the relative responsiveness of the individual neurons. We can determine the properties of encoded messages transmitted to the central nervous system (specifically, their specificity and sensitivity for certain stimuli) and estimate their response thresholds and response maxima as well as their dynamic stimulus ranges. By these means, we can gain a better understanding of the role of the sensory neurons in the regulation of the insect's behavior. For example, by examining the interaction of agonist and antagonist stimuli, we can determine the mode of action of insect repellents and attractants; by examining the structure–activity relationships (SAR) of a single class of neuron for its best stimulus, we can define the requirements for rational design of new attractants or repellents having certain desired properties. In contrast, electroantennagrams (EAGs) are antennal recordings of an uncertain genesis and include the summed potentials representing the activities of all cells responding, both excited and inhibited, as well as potentials of unknown origin. These EAGs are very useful for screening compounds for general activity. Ultimately, one must resort to single cell activity to discern the underlying sensory–behavior mechanisms.

*Odor-delivery system:* Chemical stimuli are presented in one of 2 ways. In the qualitative method, neat compounds are placed on filter paper in 10-ml syringes and the vapors manually expelled over the antenna. This method is used to screen large numbers of compounds over the antennal sensilla relatively rapidly and, in part, determines the specificity of the sensory neurons whose responsiveness is being characterized. Once we have determined the substances to which the neuron reacts, we place those substances in the quantitative delivery system.

The quantitative delivery system allows us to determine the relative sensitivity of the neuron to the compounds of interest. We also use this system to present multiple odors so we can examine the interactions of stimuli (e.g., an attractant and a repellent) with the sensory neuron. In the quantitative system, we pass air through a flask containing the test substance held at a constant temperature. The stimulus intensity is a function of the degree of saturation of the air stream calculated from the vapor pressure and temperature of the test substance and the flow rate of the saturated air stream (Ough and Stone 1961). The intensity of each stimulus is controlled by varying the flow rate of the odor air stream with individual flowmeters and metering valves. Stimulus presentation is accomplished by actuating a 3-way solenoid valve that redirects the stimulus air stream into a constant 1-liter/min flow of odor-free air, at 27°C and 50% RH, passing through a common delivery tube directed over the mosquito's antenna.

*Behavioral assay:* To correlate activity (i.e., sensitivity) of a particular receptor with the presence or absence of a specific behavior, we first determine the behavioral response of an individual female mosquito and then determine the electrophysiological responsiveness of her sensory receptors. A variety of behavioral assay systems are used in our laboratory depending upon the specific behavior and the associated sensory organs being examined. For example, we use the Feinsod-Spielman apparatus (Feinsod and Spielman 1979), dual-port olfactometers, a laminar flow wind tunnel, and small special purpose chambers. For some behaviors, we use frame-by-frame video analysis.

## RECEPTOR SYSTEMS MEDIATING MOSQUITO BEHAVIOR

The receptor systems of the antennae and maxillary palpi have been categorized according to the behavior that they mediate and the signal that they detect and encode. Some receptors may provide input for more than one behavior; examples of such receptors are presented below in discussions of specific receptors.

### Host location

a. *Lactic acid:* Lactic acid is a metabolic product common to all animals and is present in every emanation from humans (Ditmer 1961). Acree et al. (1968) identified lactic acid as an attractant for *Aedes aegypti* (Linn.) females. Lactic acid is detected by 2 classes of neurons within the grooved-peg sensilla (McIver 1970) on the antenna of both males (Davis 1977) and females (Davis and Sokolove 1976). One class of neurons is excited by lactic acid, while the other is inhibited by the substance at intensity ranges emanating from a human hand (Fig. 1A). The vertical dotted line at 27.9 mol/sec in Fig. 1 indicates the highest expected level of lactic acid evolution from a human hand (Smith et al. [1970]: recalculated from the mean of 11 human subjects [15.0 mol/sec] plus 3 SD [12.9 mol/sec]). With a series of structural analogs of lactic acid, it was found that the optimal stimulus for the lactic acid-excited neuron was a 3-carbon monocarboxylic acid with a hydroxyl or thiol group at the 2-carbon position (i.e., lactic acid and thiolactic acid). Both the D(−)- and L(+)-isomers of lactic acid are detected equally by these neurons (Davis 1988).

The detection of lactic acid is clearly important for female mosquitoes that use lactic acid to find a source of a blood meal. That both sexes of *Ae. aegypti* detect and are attracted by lactic acid is also significant because this species mates in the vicinity of a host (Hartberg 1971).

The level of sensitivity of the lactic acid-excited neuron is known to be correlated with the presence or absence of host-seeking behavior. Newly emerged *Ae. aegypti* do not host-seek until the lactic acid-excited neurons have reached a high level of sensitivity (Davis 1984b). Following a blood meal, further host-seeking behavior is inhibited by an endogenous factor (Klowden and Lea 1979b). We have shown that this humoral inhibition of behavior is correlated with a concurrent humorally mediated supression of sensitivity of the lactic acid-excited neuron (Fig. 1B) (Davis 1984a). Bowen et al. (1988) demonstrated that receptor sensitivity to lactic acid as well as host-seeking behavior is similarly depressed in *Culex pipiens* (Linn.) females in reproductive diapause. These results lead us to hypothesize that a reversible change in sensitivity in the lactic acid-excited neuron of the excitatory–inhibitory combination of lactic acid-sensitive neurons is sufficient to account for the reversible switching between host-seeking and non-host-seeking behavioral states (Davis et al. 1987).

b. *Ammonia:* In the tick, *Rhipicephalus sanguineus* (Latreille), ammonia evokes "questing behavior", that is the waving about of the tick's first pair of tarsi, on which reside its primary olfactory receptors. This extension and waving of the tick's first pair of legs accomplishes 2 functions: first, the olfactory receptors on the tick's first tarsi are analogous to an insect's antennae, and extending them puts them in a better position to detect airborne odors; and 2nd, the extended tarsi are in a better position to grasp a passing host animal. Haggart and Davis (1980) found 2 neurons associated with 2 different tarsal sensilla of these ticks that responded to ammonia over a combined intensity range of 0.2–100 $\times 10^{-9}$ mol/sec. Although no ammonia-specific sensory neuron has been reported from mosquitoes and the behavioral significance of ammonia in mosquitoes is unknown, we occasionally obtain specific electrophysiological responses to low levels of ammonia in the grooved-peg sensillum.

c. *Carbon dioxide:* Kellogg (1970) reported finding a class of neurons in the capitate sensilla on the ventral-medial aspect of the maxillary palpi of female *Ae. aegypti* that are sensitive in a dose-dependent manner to levels of $CO_2$ found in exhaled human breath (Fig. 2).

That $CO_2$ is behaviorally important in host attraction has been clearly established (Gillies 1980). It acts synergistically both with lactic acid (Mayer and James 1969) and with octenol (Kline et al. 1991). An attraction to $CO_2$ alone has also been reported from field studies. However, even though $CO_2$ alone may be associated with large increases in trap catches, there have been no independent measures of the effectiveness of $CO_2$ relative to the total mosquito population or of its effectiveness as related to the physiological state of the vector.

d. *Octenol:* 1-Octen-3-ol, isolated from ox breath, has been reported as a blood-feeding attractant for the tsetse fly, *Glossina morsitans* Westwood (Vale and Hall 1985), and an antennal receptor for this substance has been identified and characterized (Bogner 1992, den Otter and Van der Goes van Naters 1992).

In mosquitoes, 1-octen-3-ol has been demonstrated to attract certain species in the field (e.g., *Aedes taenorhynchus* (Weid.) and *Culex salinarius* Coq.) but not others (e.g., *Ae. aegypti*)

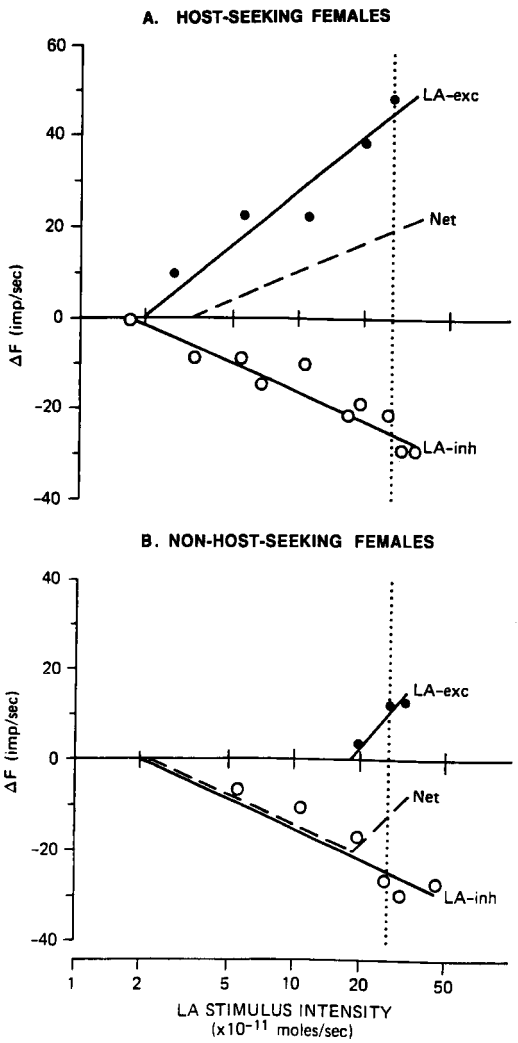


Fig. 1. Stimulus intensity–response functions for the lactic acid-excited (LA-exc) and lactic acid-inhibited (LA-inh) neurons in the antennal grooved-peg sensilla of *Aedes aegypti*: (A) from host-locating females and (B) from non-host-locating females. The vertical line at 28 mol/sec indicates the maximum expected level of lactic acid emission from a human hand.

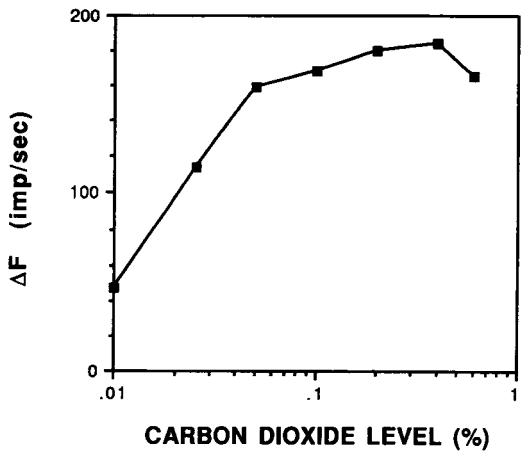


Fig. 2. Mean response of 3 neurons in the sensilla basiconica on the maxillary palpi of *Aedes aegypti* to changes in the level of carbon dioxide above background (0.04%). ΔF is the change in spike frequency.

(review by Kline 1994). However, the functional and behavioral significance of octenol may not be the same in each species sensitive to it. For example, nongravid *Ae. taeniorhynchus* are attracted to traps baited with octenol, whereas both gravid and nongravid *Cx. salinarius* are caught with octenol. In certain biting midges (*Culicoides* spp.), only females beginning their 2nd gonotrophic cycle are caught in octenol-baited traps, which suggests that some populations or strains may be autogenous in their first gonotrophic cycle. Bentley et al. (1981) reported that in the laboratory gravid *Aedes triseriatus* (Say) were weakly attracted to an oviposition site with 1-octen-3-ol.

Because those species that have a strong behavioral response to 1-octen-3-ol or other octenols have not been examined electrophysiologically, the presence or absence of a receptor in mosquitoes sensitive to one or more of these compounds has not yet been reported.

e. *Phenols:* Alkyl-phenols isolated from cattle and buffalo urine, specifically, 3- and 4-methyl, -ethyl, and *n*-propyl phenols, have been found to attract tsetse flies to an animal host (Bursell et al. 1988). In addition, den Otter and Van der Goes van Naters (1993) described 4 classes of antennal neurons of *G. morsitans* that were differentially sensitive to this group of substances. The role of phenols as host attractants in mosquitoes has not been established. Gravid *Ae. triseriatus* are attracted to an oviposition site by 4-methyl phenol and a receptor in the short, blunt A2 sensilla has been described (Bentley et al. 1982). Whether or not the mosquito could use 4-methyl phenol for both host-seeking and oviposition site location signals, depending on the insect's physiological state, is not known.

f. *Temperature:* Temperature is an important physical signal associated with animals and mosquitoes and other vector organisms can detect and orient to it and appear to use it in host location. The thermosensitive neurons are associated with the coeloconica sensilla at the tip of the mosquito's antennae and, because of internal anatomical similarity, probably in the sensilla ampullacae found along the other antennal segments as well (McIver and Hutchinson 1972). The coeloconica sensilla contain 2 types of ther-

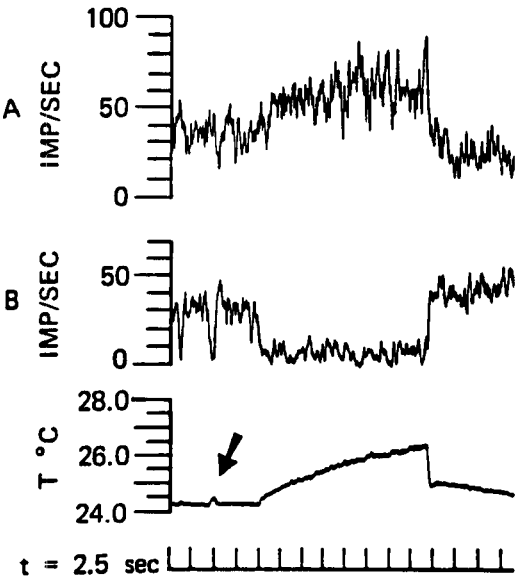


Fig. 3. Response (instantaneous frequency) of (A) the warm receptor and (B) the cold receptor in the antennal sensilla coeloconica of *Aedes aegypti* to rapid changes in temperature. The bottom trace indicates the temperature within 2 mm of the antennal receptors. The arrow indicates the response of the thermoreceptors to a pressure wave caused by someone walking in the room about 3 m away from the preparation.

mosensitive neurons, one having a positive temperature coefficient and the other a negative temperature coefficient, such that they form a differential responding pair (Fig. 3) (Davis and Sokolove 1975).

The dynamic sensitivity of the thermal receptor system of the mosquito is quite high. A rapid temperature change of 0.05°C will cause a change in spike frequency ($\Delta F$) of 4 impulses/sec in both the warm and the cold thermoreceptors for a total response of 8 impulses/sec/0.05°C (Davis and Sokolove 1975). If the activities of all 10–12 thermoreceptor sensilla per antenna are summed, the total activity would be in the range of 80–96 impulses/sec, which would suggest a very high sensitivity to very small rapid changes in temperature, as found in thermal convection currents.

Temperature changes on the order of 0.05°C within convection currents arising from a 2-kg rabbit can be detected from more than 2 m away. Compression waves produced by a body moving in air 3 m from the mosquito will cause the thermoreceptors to respond with up to 30 impulses/sec (Fig. 3, at arrow). Moisture in animal emanations travelling with the odor filament can provide thermal information to the mosquito. These observations and the dynamic sensitivity of the thermoreceptor system suggest that temperature is not as short range a host signal as many believe.

The thermal receptor pair has a low sensitivity to slow changes in ambient temperature: change in spike frequency is 4–5 impulses/sec/°C ($\Delta F/\Delta T$) over the temperature range 18–40°C for each type of thermoreceptor (Davis and Sokolove 1975). These increases in tonic firing rates as a function of the ambient temperature are seen in both the warm and cold receptors and probably provide information that the mosquito is within the optimal temperature range for behavioral and physiological activity. It is unlikely that these tonic responses could encode for the absolute ambient temperature, because the signal-to-noise ratios and the stimulus and response uncertainties are high enough that the tonic $\Delta F/\Delta T$ is too small to permit discrimination between ambient temperature levels differing by less than 4°C. The reader is referred to Smith et al. (1983) and their estimate of the information transmission capacity of tarsal sugar receptors of *Phormia regina* (Meigen).

g. *Humidity:* Although Kellogg (1970) described a humidity receptor in the A3 grooved-peg sensillum, subsequent studies in our laboratory failed to confirm this finding and, in fact, demonstrated a totally different function for the neurons within this sensillum—specifically, the detection of lactic acid (see above). Closer scrutiny of Kellogg's dose–response functions for humidity suggest that the cells he identified, as well as the other antennal receptors, are merely moisture dependent (i.e., if the humidity falls, they lose sensitivity). However, we know warm moist air attracts mosquitoes better than warm dry air probably because moist air carries temperature information to which the mosquito is very sensitive.

*Summary:* To date, we have identified and characterized receptors for lactic acid, $NH_3$, $CO_2$, cresols, and temperature, but not all of these may be found on all species of mosquitoes. There most certainly are other receptors we have not yet found, in part because no one has identified the stimuli to which they respond. For example, about 50–60% of a population of avid *Ae. aegypti* can be attracted using an artificial host odor consisting of warm, moist air (50% RH at 27°C) with lactic acid ($20 \times 10^{-11}$ mol/sec) and $CO_2$ (4.0%); however, the responding females do not probe (<3%). Using the human hand as bait, we can attract 80–95% of the same population, and 99% of them will probe *without* contacting the hand. Thus, probing is mediated by an odor that has yet to be identified (Davis and Bowen, unpublished data).

Some reported host-attractant candidates—such as alanine and lysine (Brown and Carmi-

Table 1. Responses[1] of antennal chemosensory neurons to odors putatively associated with plant resource location by mosquitoes.

| Species | Sensilla type[2] | Terpenes[3] | | Green plant volatiles[4] | Fatty acid esters[5] |
|---|---|---|---|---|---|
| | | Bicyclic | Monocyclic and acyclic | | |
| *Aedes aegypti* (Lacher 1967) | A1 | | strong | NR | weak–moderate |
| | A2-L | | moderate–strong (terpineol) | NR | NR |
| *Culex pipiens* (Bowen 1992a) | A2-L | strong (thujone) | NR | NR | NR |
| | | strong | NR | NR | strong |
| *Culiseta* spp. (Bowen, unpublished data) | A2-L | strong (thujone) | NR | NR | NR |
| | A2-S | NR | NR | strong | NR |

[1] Qualitative response (to neat stimulus): strong = >30 impulses/sec, moderate ~15 impulses/sec, weak = <15 impulses/sec; NR = no response; quantitative response to thujone over a stimulus range of 0.4–7.2 × $10^{-7}$ mol/sec.
[2] Sensilla trichoidea of average varying lengths: A1 ~55 μm; A2-L ~35.3 μm; A2-S ~21.7 μm.
[3] Bicyclic terpenes included thujone, verbenone, and a-pinene; mono- and acyclic terpenes included limonene, terpineol, menthone, eugenol, thymol, citral geraniol, nerol, and citronellol.
[4] Hexanylacetate, hexanal, 1-hexanol, (*cis*)-3-hexen-1-ol, and (*trans*)-3-hexen-1-ol.
[5] Ethyl propionate, methyl propionate, methyl butyrate, and ethyl acetate.

chael 1961)—are unlikely because their vapor pressures at skin temperatures are so low that they are not found in the vapor phase at levels detectable by the insect's olfactory receptors.

### Plant location and feeding

Adult nutritional status has been shown clearly to affect many aspects of mosquito behavior. Both larval nutrition and adult nectar feeding are important in establishing and maintaining adequate nutritional reserves and meeting essential nutritional requirements. Nectar feeding is a common behavior in many vector mosquitoes (Yuval 1992). All males are obligatory plant juice feeders. Females of most species will feed from plants as a carbohydrate resource and some feed on plants to develop eggs and/or increase fecundity.

Our understanding of the stimuli and the receptors that underlie nectar resource location is in its infancy. Three major problems have been encountered with plant-relates stimuli and with the receptors that encode for these stimuli. First, the range of classes of chemical constituents and the numbers of compounds within each chemical class associated with plants on which mosquitoes feed are almost overwhelmingly large. Second, identification of the plants on which mosquitoes feed has barely begun; very few are known, and even fewer have been analyzed with respect to the volatile components that attract mosquitoes. Third, we have not yet been able to morphologically differentiate the various physiological classes of sensory neurons that must be on the antenna to discriminate between nectar resources of choice. In spite of these problems we are making some progress in identifying and characterizing plant-odor receptor systems. Careful measurement of sensillum length, mapping of sensillar position on the antenna, and scanning electron microscopy examination of the sensillum after electrophysiological responses have been obtained will help to differentiate the various receptor types in the future (Davis 1974).

Although the primary focus over the years has been on the sensory responses to host-related and oviposition site-related odors, the responses of several types of sensilla to plant-related odors have been recorded. These responses are summarized in Table 1 according to the type of sensillum and the mosquito species from which the responses were obtained. As is evident, the data are extremely sparse for this important behavior.

Of the 2 neurons present in the longer A2 type (McIver 1978) sensillum (mean length 35.3 μm) of *Cx. pipiens,* the neuron having a low mean spontaneous activity rate (0.4 impulse/sec) was found to be thujone-specific. Behaviorally, thujone appears to be a close-range airborne stimulus that evokes probing but does not attract over a distance in an olfactometer (Bowen 1992b). Lacher (1967) reported a sensory neuron within an A2-type sensillum that appeared to be terpineol-specific. Interestingly, only in *Culiseta incidens* (Thompson) have we been able to find a class of neurons that respond to certain green plant odors such as hexanal, 1-hexanol, and *cis-*

Table 2. Antennal sensilla sensitive to oviposition attractants.

| Species | Attractant[1] | Sensilla | Reference[2] |
|---|---|---|---|
| *Culex quinquefasciatus* | egg raft pheromone | unknown | |
| | 3-methyl-indole | unknown | |
| *Culex pipiens* | egg raft pheromone | A2, short sharp | Bowen (unpublished) |
| *Culex tarsalis* | 2-BOE, o-cresol | A2, short blunt | |
| | 2-BOE, 4-MCH | A2, short sharp | Davis (unpublished) |
| *Aedes triseriatus* | p-cresol | A2, short blunt | |
| | 4-MCH | A2, short sharp | Bentley et al. (1982) |
| | 1-octen-3-ol | unknown | |
| *Aedes aegypti* | fatty acid esters, e.g., EP | A2, short sharp | Davis (1976) |
| | cresols | A2, short blunt | Davis (unpublished) |
| *Anopheles albimanus* | o-cresol | A2, short blunt | |
| | EP (and 4-MCH) | A2, short sharp | Bowen (unpublished) |
| *Anopheles stephensi* | 2-BOE, cresols | A2, short blunt and A2, short sharp | Davis (unpublished) |

[1] 2-BOE = 2-butoxyethanol (Ikeshoji 1968), 4-MCH = *cix*-4-methylcyclohexanol (Bentley et al. 1982), EP = ethyl propionate.
[2] References refer to the identification of a responding sensillum type.

and *trans*-3-hexen-1-ol (Bowen, unpublished data).

### Oviposition site location

In all species that have been examined to date, the neurons sensitive to oviposition site-related odors have been found in either the short, sharp or the short, blunt A2 sensilla trichoidea (or in both), depending on the species of mosquito (Table 2).

From the relative diversity of oviposition site-related compounds, oviposition site selection appears to have a certain degree of species specificity. Most of the attractive compounds isolated from oviposition sites appear to be bacterial or fungal metabolites. To date, only *Culex quinquefasciatus* Say is known to produce a pheromone from its egg rafts that attracts conspecific gravid females (Laurence and Pickett 1985). This mosquito also responds to 3-methyl-indole isolated from Bermuda grass infusion. These 2 compounds act synergistically in attracting gravid *Cx. quinquefasciatus* (Millar et al. 1992). *Culex pipiens* possesses neurons sensitive to the egg raft pheromone in its short, sharp A2 sensilla trichoidea (Bowen, unpublished data), but the behavioral significance of this sensitivity has yet to be determined.

Bentley et al. (1982) found sensory neurons on the antennae of *Ae. triseratus* that are sensitive to p-cresol (4-methylphenol) and a structural analog, *cis*-4-methyl cyclohexanol (4-MCH), over the range 1–50 × $10^{-7}$ mol/sec. Because both p-cresol and 4-MCH evoked oviposition attraction in this mosquito, they were thought to be functional analogs. However, this is not the case, because p-cresol is detected by neurons in the short, blunt A2 sensilla and 4-MCH by neurons in the short, sharp A2 sensilla. This functional dissimilarity was only discovered through electrophysiological examination of the responses of neurons within single sensilla. Interestingly, p-cresol is attractive to gravid *Ae. triseriatus*, whereas the o- and m-cresols are contact stimulants for oviposition in this species (Bentley et al. 1981). *Anopheles albimanus* Wied. detects airborne o-cresol in its short, blunt sensilla trichoidea (Bowen, unpublished data) but the behavioral relevance of this finding is unknown. Similarly, the significance of the behavioral and sensory responsiveness to methyl and ethyl fatty acid esters by gravid *Ae. aegypti* is in question because these substances have never been isolated from natural ovisites (Bentley and Day 1989).

## PRACTICAL APPLICATION OF SENSORY-PHYSIOLOGICAL MECHANISMS TO THE MANIPULATION OF MOSQUITO BEHAVIOR

*Rational design of attractants:* The strategy for the rational design of biologically active substances (e.g., pharmaceuticals and pesticides) can be applied to the design of newer and better attractants, deterrents (of feeding, oviposition, etc.), and repellents for use against disease vector arthropods. Through a knowledge of the SARs of a receptor and its ligand, we can specify to a synthetic chemist the precise requirements of the agonists or antagonists of that system and the

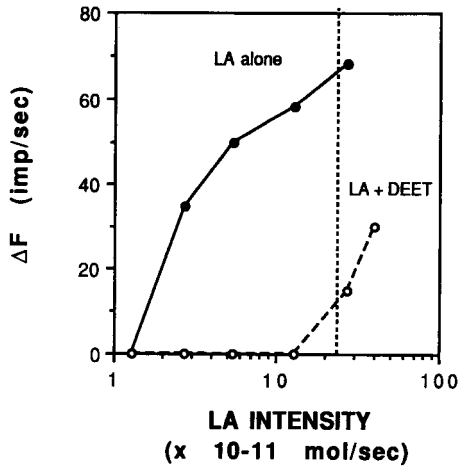


Fig. 4. Response of a single lactic acid-excited neuron from *Aedes aegypti* to lactic acid alone (●) and to lactic acid plus the insect repellent deet, at $10.5 \times 10^{-7}$ mol/s (O). The vertical dotted line is the maximum expected level of lactic acid emission from a human hand (Davis et al. 1987, Fig. 1; reprinted with permission from ICIPE Science Press).

properties the desired compound must have. The chemist then has a rational basis for designing compounds to meet the receptor's requirements and our needs.

In studies in our laboratory, we presented the lactic acid sensitive neurons with a series of analogs of lactic acid having systematic variations in their structures, our goal being to determine the SAR of this receptor system (Davis 1988). These structural variations included the number, type, and location of the functional groups as well as the length of the basic carbon chain. The results indicated that the best (i.e., most effective) stimulus for the lactic acid receptors was a 3-carbon monocarboxylic acid with a substitution at C2 consisting of -OH and -SH (nearly equal in effectiveness) and -Cl or -Br (equal effectiveness but 10 times less than that of -OH or -SH). All of the other analogs were less effective (1,000-fold or more) in exciting the lactic acid sensory neurons. Thus, lactic acid was the most effective stimulus for this type of neuron. During this study, we presented oxamic and oxalic acids during the presentation of lactic acid. These 2-carbon analogs of lactic acid interfered with the response of the lactic acid-excited neuron to lactic acid, even though neither one elicited a response when presented alone (Davis, unpublished data). These results could form the basis for developing an inhibitor that has a higher binding affinity for the receptor than lactic acid but, like oxamic and oxalic acids, is ineffective in causing a response and thus interferes with the mosquito's ability to locate a host until the blocked receptor is replaced by normal membrane receptor turnover.

*Mode of action of repellents:* Another example of how coupling sensory physiology with behavior can be used to practical advantage is in determining the mode of action of a biologically active substance. Although the example presented here is for the repellent deet, the same approach can be used for any behaviorally active substance.

Electrophysiological studies have shown that deet interferes with the transmission of spikes from a lactic acid-excited neuron stimulated with lactic acid (Fig. 4) (Davis and Sokolove 1976, Davis 1985, Davis et al. 1987).

Examining the flight patterns of individual female mosquitoes in a wind tunnel we observed the following. Mosquitoes exposed to repellent either alone or with host odor fly slower than those exposed to host odor alone or odor-free conditions. The angles of flight path segments relative to the wind direction correlate with odor condition (Table 3). Females flying upwind in host odor alone make most of their turns within angles <45° from the upwind direction, whereas those flying in odor-free air or repellent-laden air (either with or without host odor) make significantly more turns with angles of 45–90° or >90°

Table 3. Percent of flight path segments within 3 ranges of flight path angle to the wind.

| Condition | $n$ | Angle to the wind[1] 0–45° | 45–90° | >90° | No. segments[2] |
|---|---|---|---|---|---|
| Host odor | 18 | 97.8 ± 6.8 | 2.2 ± 6.8 | 0.0 ± 0.0 | 3.8 |
| Odor free | 17 | 86.8 ± 19.9* | 8.0 ± 15.3 | 5.2 ± 10.5* | 4.4 |
| Repellent | 17 | 82.9 ± 19.8* | 8.5 ± 12.2 | 8.5 ± 12.1* | 7.0** |
| Host odor + repellent | 12 | 83.3 ± 16.4* | 10.8 ± 10.6* | 6.0 ± 9.1* | 7.2** |

[1] Mean % (±SD) of flight path segments of individual female mosquitoes.
[2] Mean number of flight path segments per female.
* Significantly different from Host odor condition at $P < 0.05$ (Student's $t$-test).
** Significantly different from both Host odor and Odor free conditions at $P < 0.05$ (Student's $t$-test).

from the upwind direction. The combination of the depression of lactic acid sensitivity and the similarity of the behavioral flight characteristics in odor-free and repellent-laden air suggests that deet is not a repellent per se, that is it does not cause the female to orient away from the source. Instead, deet appears to render the female anosmic to an important host attractant (lactic acid).

In a manner similar to its inhibition of host attraction, deet also inhibits the attraction of gravid female *Ae. aegypti* to oviposition site stimuli. Deet also interferes with sensory detection of the oviposition site attractant ethyl propionate (Kuthiala et al. 1992). This result, together with the interference with lactic acid detection, suggests that deet does not act at the membrane receptor–ligand interface but at some more general site associated with the action potential excitation process.

## SUMMARY

Because mosquitoes orient to biologically important resources such as hosts, nectar sources, and oviposition sites via airborne chemical and physical signals detected by peripheral sensory systems, and because mosquitoes' responses to relevant environmental signals differ depending upon (a) species and (b) physiological state, a fundamental understanding of the sensory processes that mediate and regulate the critical behaviors of the insect is necessary for the rational development of newer and better materials and strategies for the control of these important organisms.

## ACKNOWLEDGMENTS

Some of the work reported herein was sponsored by grants from National Institute for Allergy and Infectious Diseases to M. F. Bowen (AI23336) and E. E. Davis (AI21267).

## REFERENCES CITED

Acree, F., R. B. Turner, H. K. Gouck, M. Beroza and N. Smith. 1968. L-lactic acid: a mosquito attractant isolated from humans. Science 161:1346–1347.

Bentley, M. D. and J. F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. Annu. Rev. Entomol. 34:401–421.

Bentley, M. D., I. N. McDaniel and E. E. Davis. 1982. Studies of 4-methylcyclohexanol: an *Aedes triseriatus* (Diptera: Culicidae) oviposition attractant. J. Med. Entomol. 19:589–592.

Bentley, M. D., I. N. McDaniel, M. Yatagai, H. P. Lee and P. Maynard. 1981. Oviposition attractants and stimulants of *Aedes triseriatus* (Say) (Diptera: Culicidae). Environ. Entomol. 10:186–189.

Bogner, F. 1992. Response properties of $CO_2$-sensitive receptors in tsetse flies (Diptera: *Glossina palpalis*) Physiol. Entomol. 17:19–24.

Bowen, M. F. 1990. Post-diapause sensory responsiveness in *Culex pipiens*. J. Insect Physiol. 36:923–929.

Bowen, M. F. 1991. The sensory physiology of host-seeking behavior in mosquitoes. Annu. Rev. Entomol. 36:139–158.

Bowen, M. F. 1992a. Patterns of sugar feeding in diapausing and nondiapausing *Culex pipiens* (Diptera: Culicidae) females. J. Med. Entomol. 29:843–849.

Bowen, M. F. 1992b. Terpene-sensitive receptors in female *Culex pipiens* mosquitoes: electrophysiology and behaviour. J. Insect Physiol. 38:759–764.

Bowen, M. F., E. E. Davis and D. A. Haggart. 1988. A behavioural and sensory analysis of host-seeking behaviour in the diapausing mosquito *Culex pipiens*. J. Insect Physiol. 34:805–813.

Brown, A. W. A. and A. G. Carmichael. 1961. Lysine and alanine as mosquito attractants. J. Econ. Entomol. 54:317–324.

Bursell, E., A. J. E. Gough, P. S. Beevor, A. Cork, D. R. Hall and G. A. Vale. 1988. Identification of components of cattle urine attractive to tsetse flies, *Glossina* spp. (Diptera: Glossinidae). Bull. Entomol. Res. 78:281–292.

Davis, E. E. 1974. Identification of antennal chemoreceptors of the mosquito, *Aedes aegypti*: a correction. Experientia 30:1282.

Davis, E. E. 1976. A receptor sensitive to oviposition site attractants on the antennae of the mosquito, *Aedes aegypti*. J. Insect Physiol. 22:1371–1376.

Davis, E. 1977. Response of the antennal receptors of the male *Aedes aegypti* mosquito. J. Insect Physiol. 23:613–617.

Davis, E. E. 1984a. Regulation of sensitivity in the peripheral chemoreceptor systems for host-seeking behaivour by haemolymph-borne factor in *Aedes aegypti*. J. Insect Physiol. 30:179–183.

Davis, E. E. 1984b. Development of lactic acid-receptor sensitivity and host-seeking behaviour in newly emerged female *Aedes aegypti* mosquitoes. J. Insect Physiol. 30:211–215.

Davis, E. E. 1985. Insect repellents: concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Culicidae). J. Med. Entomol. 22:237–243.

Davis, E. E. 1988. Structure–response relationship of the lactic acid-excited neurones in the antennal grooved-peg sensilla of the mosquito *Aedes aegypti*. J. Insect Physiol. 34:443–449.

Davis, E. E. and P. G. Sokolove. 1975. Temperature responses of antennal receptors of the mosquito, *Aedes aegypti*. J. Comp. Physiol. 96:223–236.

Davis, E. E. and P. G. Sokolove. 1976. Lactic acid-sensitive receptors on the antennae of the mosquito, *Aedes aegypti*. J. Comp. Physiol. 105:43–54.

Davis, E. E., D. A. Haggart and M. F. Bowen. 1987. Receptors mediating host-seeking behaviour in mosquitoes and their regulation by endogenous hormones. Insect Sci. Appl. 8:637–641.

den Otter, C. J. and W. M. Van der Goes van Naters. 1992. Single cell recording from tsetse (*Glossina m.*

*morsitans*) antennae reveal olfactory, mechano- and cold receptors. Physiol. Entomol. 17:33–42.

den Otter, C. J. and W. M. Van der Goes van Naters. 1993. Responses of individual antennal olfactory cells of tsetse flies (*Glossina m. morsitans*) to phenols from cattle urine. Physiol. Entomol. 18:43–49.

Ditmer, D. S. (editor). 1961. Biological handbooks: blood and other body fluids. FASEB, Washington, DC.

Feinsod, F. M. and A. Spielman. 1979. An olfactometer for measuring host-seeking behavior of female *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 15:282–285.

Gillies, M. T. 1980. The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae). Bull. Entomol. Res. 70:525–532.

Haggart, D. A. and E. E. Davis. 1980. Ammonia-sensitive neurones on the fist tarsi of the tick, *Rhipicephalus sanguineus*. J. Insect Physiol. 26:517–523.

Hartberg, W. K. 1971. Observations on the mating behavior of *Aedes aegypti* in nature. Bull. W.H.O. 45:847–850.

Ikeshoji, T. 1968. Studies on mosquito attractants and stimulants V. Gas chromatographic separation of the attractants for oviposition of *Culex pipens fatigans* from the field water. Appl. Entomol. Zool. 3:176–188.

Kellogg, F. E. 1970. Water vapour and carbon dioxide receptors in *Aedes aegypti*. J. Insect Physiol. 16:99–108.

Kline, D. L. 1994. Olfactory attractants for mosquito surveillance and control: 1-octen-3-ol. J. Am. Mosq. Control Assoc. 10:280–287.

Kline, D. L., J. R. Wood and J. A. Cornell. 1991. Interaction effects of 1-octen-3-ol and carbon dioxide on mosquito (Diptera: Culicidae) surveillance and control. J. Med. Entomol. 28:254–258.

Klowden, M. J. and A. O. Lea. 1979a. Abdominal distention terminates subsequent host-seeking behaviour of *Aedes aegypti* following a blood meal. J. Insect Physiol. 25:583–585.

Klowden, M. J. and A. O. Lea. 1979b. Humoral inhibition of host-seeking in *Aedes aegypti* during oocyte maturation. J. Insect Physiol. 25:231–235.

Kuthiala, A., R. Gupta and E. E. Davis. 1992. The effect of repellents on the responsiveness of the antennal chemoreceptors for oviposition of the mosquito *Aedes aegypti*. J. Med. Entomol. 29:639–643.

Lacher, V. 1967. Elektrophysiologische untersuchungen an einzelnen Geruchsrezeptoren auf den Entennen weiblicher Moskitos (*Aedes aegypti* L.). J. Insect Physiol. 13:1461–1470.

Laurence, B. R. and J. A. Pickett. 1985. An oviposition attractant pheromone in *Culex quinquegfasciatus* Say (Diptera: Culicidae). Bull. Entomol. Res. 75:283–290.

Mayer, M. S. and J. D. James. 1969. Attraction of *Aedes aegypti* (L.): responses to human arms, carbon dioxide, and air currents in a new type of olfactometer. Bull. Entomol. Res. 58:629–642.

McIver, S. B. 1970. Comparative study of antennal sense organs of female culicine mosquitoes. Can. Entomol. 102:1258–1267.

McIver, S. 1978. Structure of sensilla trichodea of female *Aedes aegypti* with comments on innervation of antennal sensilla. J. Insect Physiol. 24:383–390.

McIver, S. B. and S. A. Hutchinson. 1972. Coeloconic sensilla on the antennae of the yellow fever mosquito, *Aedes aegypti* (L.). Experientia 28:323.

Millar, J. G., J. D. Chaney and M. S. Mulla. 1992. Identification of oviposition attractants for *Culex quinquefasciatus* from fermented Bermuda grass infusions. J. Am. Mosq. Control Assoc. 8:11–17.

Ough, C. S. and H. Stone. 1961. An olfactometer for rapid and critical odor measurement. J. Food Sci. 26:452–456.

Smith, C. N., N. Smith, H. K. Gouck, D. E. Weidhaas, I. H. Gilbert, M. S. Mayer, B. J. Smittle and A. Hofbauer. 1970. L-lactic acid as a factor in the attraction of *Aedes aegypti* (Diptera: Culicidae) to human hosts. Ann. Entomol. Soc. Am. 63:760–770.

Smith, D. V., E. Bowdan and V. G. Dethier. 1983. Information transmission in tarsal sugar receptors of the blowfly. Chem. Sens. 8:81–100.

Smith, J. J. B., B. K. Mitchell, B. M. Rolseth, A. T. Whitehead and P. J. Albert. 1990. SAPID tools: microcomputer programs for analysis of multi-unit nerve recordings. Chem. Sens. 15:253–270.

Sutcliffe, J. F. 1994. Sensory bases of attractancy: morphology of mosquito olfactory sensilla—a review. J. Am. Mosq. Control Assoc. 10:309–315.

Takken, W. 1991. The role of olfaction in host-seeking of mosquitoes: a review. Insect Sci. Appl. 12: 287–295.

Vale, G. A. and D. R. Hall. 1985. The role of 1-octen-3-ol, acetone and carbon dioxide in the attraction of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), to ox odour. Bull. Entomol. Res. 75:209–217.

Yuval, B. 1992. The other habit: sugar feeding by mosquitoes. Bull. Soc. Vector Ecol. 17:150–156.