6

Evolution of Pheromonal Specificity in Insect Chemoreceptors

Richard W. Mankin

U.S. Department of Agriculture, Gainesville, Florida

I. INTRODUCTION

Chemosensation, the most ancient of the senses, plays an important role in the orientation of insects to food and potential mates. The importance of this role is reflected in the diversity of numbers and types of chemoreceptors that have evolved on the antennae, mouthparts, legs, ovipositor, and epipharynx (Chapman, 1982). Notable examples are (1) the pheromone-sensitive olfactory receptor, specialized to detect sexual odors emitted by conspecifics, and (2) the contact (taste) chemoreceptor of a phytophagous insect, specialized to detect specific host and nonhost plant compounds. In general, these two chemoreceptors lie at opposite ends on a generalist-specialist spectrum of response specificity. Pheromone receptor neurons usually respond to only a few compounds that resemble their key pheromone components (e.g., Mayer and Mankin, 1985). Contact chemoreceptors tend to be generalists (Frazier, 1986), responding to a large number of different sugars, salts, or alkaloids. Exceptions to this rule do occur, however. Some insects have relatively unspecific pheromone receptor neurons (e.g., Hansson, 1988), and some contact chemoreceptor neurons in caterpillars are specialists for detecting certain secondary plant compounds (Schoonhoven and Dethier, 1966; Schoonhoven, 1967; Dethier and Crnjar, 1982). This chapter addresses some

questions about the evolution of chemoreceptor neurons that are specialists in the detection of key stimulus chemicals. A number of such questions have already been raised with respect to the coevolution of insect herbivores and host plants (Dethier, 1980). Here the problem is extended to consider the coevolution between senders and receivers in pheromone communication systems.

In many moth species, the antennae of male (and in some cases female) adults contain sensilla with olfactory receptor neurons that respond selectively to separate components of the pheromone blend emitted by conspecific females. Other neurons in these sensilla respond to components emitted by females of other species. The blend of pheromone components is different for each species, but blends emitted by closely related species tend to be more similar than those emitted by distantly related species (Roelofs and Brown, 1982; Renou et al., 1988; Horak et al., 1988). Several moth species in the subfamily Plusiinae (Figure 1), share (*Z*)-7-dodecen-1-ol acetate (Z7-12:Ac) as a major sex pheromone component (Steck et al., 1982) and share a number of minor components as attractant or inhibitory chemicals (Table 1, Figure 2). Three of these species, the cabbage looper [*Trichoplusia ni* (Hübner)], the soybean looper [*Pseudoplusia includens* (Walker)], and the celery looper [*Anagrapha falcifera* (Kirby)] are of particular interest because their geographic distributions overlap (Figure 3) and they are somewhat cross-attractive

TABLE 1 Pheromone Blends of *A. falcifera*, *T. ni*, and *P. includens*[a]

Chemical	*T. ni* [b]	*P. includens* [c]	*A. falcifera* [d]
Z7-12:Ac	+a	+a	a
Z5-12:Ac	+a	−i	
Z7-14:Ac	+a	−	*i*
Z9-14:Ac	+a	−i	
11-12:Ac	+a	+a	
12:Ac	+a	+a	
Z7-12:Pro	−	+a	
Z7-12:But	−	+a	
Z7-12:OH	−i	−	a

[a]A 12 or 14 indicates the number of carbon atoms in the backbone of the molecule, Z indicates a cis double-bond at the specified carbon, and Ac, OH, Pro, and But are acetate, alcohol, propionate, and butyrate moieties, respectively, attached to the first carbon. A + or − indicates the presence or absence in the blend; a or i indicates behavioral attraction or inhibition.

[b]From Bjostad et al. (1984); Linn et al. (1984).

[c]From Linn et al. (1988).

[d]From Steck et al. (1979b), based on captures by pheromone traps.

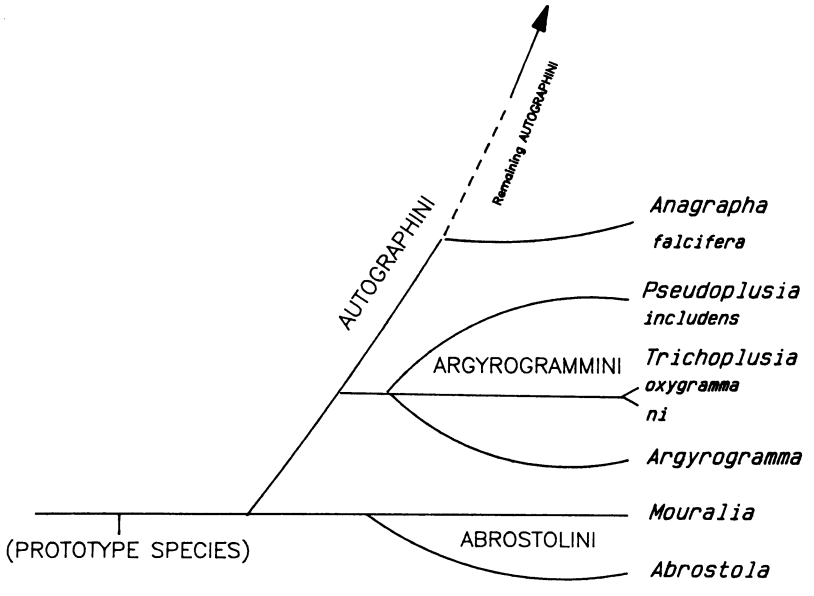

FIGURE 1 Phylogenetic tree (Eichlin and Cunningham, 1978) showing the ancestry of the three Plusiinae moth species in the study: *Anagrapha falcifera, Pseudoplusia includens,* and *Trichoplusia ni*. The tropical ancestors of these three species diverged into three tribes, of which Abrostolini is the most primitive and Autographini the least primitive. *P. includens* and *T. ni* are in the same tribe, Agryrogrammini, so they are more closely related to each other than to *A. falcifera*.

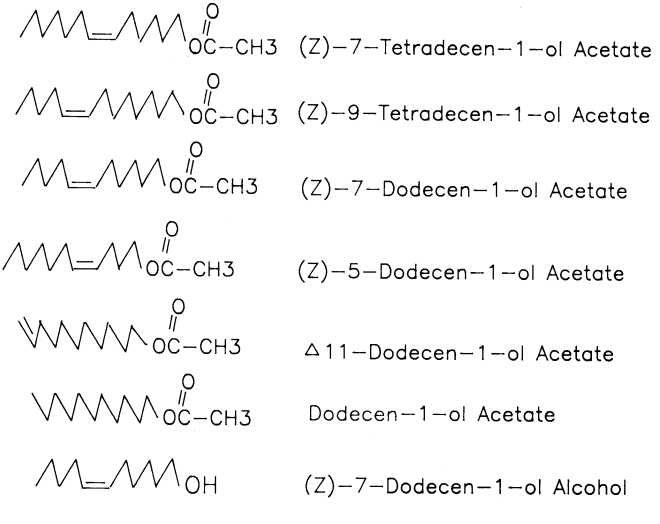

FIGURE 2 Chemical structure of pheromone components identified for *T. ni, P. includens,* and *A. falcifera*.

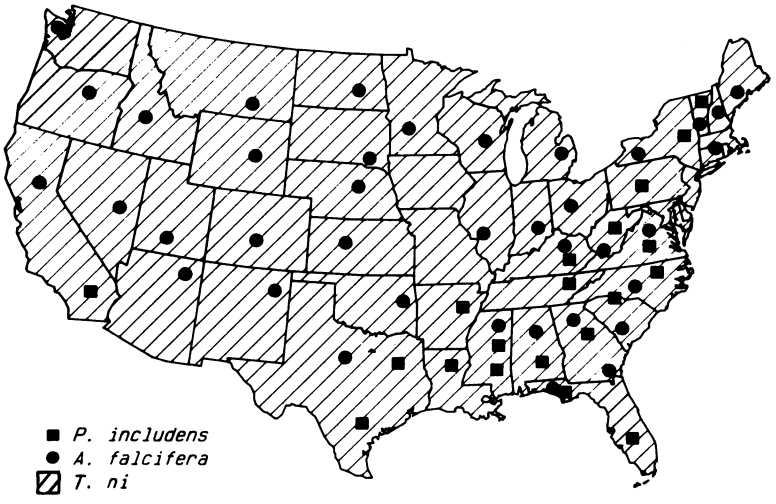

FIGURE 3 Distribution of *A. falcifera, P. includens,* and *T. ni* in the United States. *T. ni* occurs throughout the United States and has the largest population. *A. falcifera* occurs throughout the United States except in desert areas and south Florida. *P. includens* occurs in the Southwest and the Eastern Seaboard. Extensive areas of overlapping populations occur.

to each other (Kaae et al., 1973; Leppla, 1983). Consequently, these species are probably under selection to maintain reproductive isolation by changes in their pheromone communication systems. This makes them excellent candidates for a study of potential mechanisms by which pheromone senders and receivers coevolve in closely related species. Surveys of responses by pheromone receptor neurons on the antennae of *T. ni* (Mayer and Mankin, 1987) and *P. includens* (Grant et al., 1988) have been published recently. To enable further evolutionary comparisons, responses of pheromone-sensitive chemoreceptors on *A. falcifera* antennae were recorded to the same pheromone components as in the two previous studies.

II. MATERIALS AND METHODS

Adult male *A. falcifera*, aged 2-3 days after eclosion, were obtained from a colony started by Dr. Peter Landolt of this laboratory with local wild stock in 1987. The small size of the colony limited the number of males that could be tested, so this should be considered only a preliminary survey of receptor neuron types rather than a comprehensive, quantitative study. The purities and sources of the chemicals were (*Z*)-7-dodecen-1-ol acetate, no detectable

impurities, from Dr. B. Leonhardt, Insect Chemical Ecology Laboratory, Beltsville, MD; Δ-11-dodecen-1-ol acetate (11-12:Ac), 99.4%, from Dr. S. Voerman, Institute for Pesticide Research, Wageningen, The Netherlands; dodecen-1-ol acetate (12:Ac), 98.8%, from Pfalz and Bauer Corp.; (*Z*)-7-dodecen-1-ol (Z7-12:OH), no detectable impurities, from Dr. J. Tumlinson of this laboratory; and (*Z*)-5-dodecen-1-ol acetate (Z5-12:Ac), 98.3%, (*Z*)-7-tetradecen-1-ol acetate (Z7-14:Ac), 99.2%, and (*Z*)-9-tetradecen-1-ol acetate (Z9-14:Ac), 99.2%, from Farchan Corp. The analyses of purity were performed by Dr. R. Doolittle and R. Heath of this laboratory using capillary gas chromatography.

Electrophysiological recordings were obtained by inserting a tungsten electrode into the base of a sensillum and inserting a second electrode into the lumen of the distal portion of the antenna. The electrical potentials from the electrodes were amplified by a Grass P15 high-impedance preamplifier and transmitted to the 10 Hz, 12 bit Analog-Digital register of a PDP-11/23 microcomputer. User-written software stored the potentials on disk and classified the action potentials (spikes) elicited from the two neurons in each sensillum (Mankin et al., 1987). Examples of spike records generated by this procedure are found in Mankin et al. (1987) and Grant et al. (1989).

The test stimuli were delivered from glass tube dispenser assemblies (Mayer et al., 1987) that led into a stimulus delivery system described in Grant et al. (1989). Until stimulus onset, the antenna was bathed in a stream of clean carrier air at 1200 ml/minute. A separate airstream flowed concurrently through the glass tube dispenser assembly at 200 ml/minute into a vacuum pulling 250 ml/minute. When the valve was closed at stimulus onset, the stimulus stream proceeded into a glass chamber, mixed with the carrier air, and passed to the antenna. The dispensers were dosed by diluting a specified quantity (usually between 0.001 and 1.0 μg) of a pheromone component or blend of components in hexane and coating the mixture over the inside of the dispenser assembly while the hexane evaporated. The concentrations that passed over the antenna were calculated by dividing the emission rates, listed in Mayer et al. (1987), by the 1400 ml/minute mixture volume. The stimulus period was 3 s, and the spike frequencies listed in Table 1, and the figures were calculated as means over the 3 s intervals. The spontaneous activity was recorded before the first stimulus and also for 3 and 4 s periods immediately preceding and following stimulation, respectively. Stimuli were spaced about 5 minutes apart to avoid effects of adaptation.

The order of presentation was standardized on the basis of an initial survey (about 10 sensilla), which established that most of the sensilla had one neuron that responded to Z7-12:Ac and a second that responded either to Z7-12:OH or to Z5-12:Ac at dispenser doses of 0.01 μg or higher. (A dispenser dose of 1 μg emits Z7-12:Ac at approximately the same rate as a *T. ni* sex pheromone gland.) No responses were observed to 12:Ac, 11-12:Ac, or Z7-14:Ac.

Because the preparations often were viable for only 15-30 minutes, only four to six stimuli could be presented before the responses began to degrade and the presentations were standardized to quickly determine the specificities of the neurons. A blend of 0.1 μg each of 12:Ac, 11-12:Ac, and Z7-14:Ac was presented as the first stimulus. The second stimulus was a 0.032 μg dose of Z7-12:Ac. If there was no response, the next stimulus was 0.1 μg Z9-14:Ac; otherwise it was 0.1 μg Z5-12:Ac and then 0.1 μg Z7-12:OH. Once the specificity was determined, responses were recorded to a series of increasing doses of the active component. A total of 21 sensilla were tested by this procedure. About 50 others were surveyed only to determine whether they responded to Z9-14:Ac.

III. RESULTS AND DISCUSSION

A. Response Specificities of *A. falcifera* Receptor Neurons

Three different sensillum types were found on the *A. falcifera* antenna (Figure 4). One contained two neurons that responded separately to Z7-12:Ac and

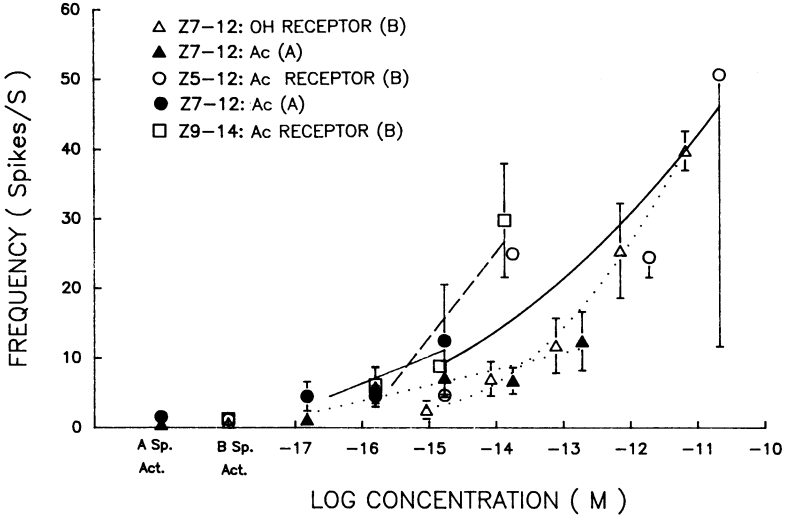

FIGURE 4 Responses of *A. falcifera* receptor neurons to the pheromone components listed in Figure 2. Responses from the A (larger amplitude) neurons are indicated by dark symbols and the B (smaller-amplitude) neurons by open symbols. The type of sensillum containing the neuron is indicated by symbol type and line type (dotted lines and triangles for sensillum found in all three species, solid lines and circles for sensillum with Z5-12:Ac receptor neuron, and dashed line for sensillum with Z9-14:Ac receptor neuron). Spontaneous activities are shown at the left of the figure.

Z7-12:OH (10 of 21 sensilla surveyed, Figure 5). The spike amplitudes and spontaneous activities (1.5 ± 0.8 spikes per s for the Z7-12:Ac-sensitive neuron and 0.6 ± 0.4 spikes per s for the Z7-12:OH-sensitive neuron) resembled those for neurons of similar specificities in *T. ni* (Mankin et al., 1987) and *P. includens* (Grant et al., 1988). The two other sensillum types, however, contained neurons whose responses were different from those in any sensillum found previously in either *T. ni* or *P. includens*. The first sensillum type contained two neurons that responded separately to Z7-12:Ac (0.7 ± 0.4 spikes per s spontaneous activity) and Z5-12:Ac (0.9 ± 0.6 spikes per s spontaneous activity, 7 of 21 sensilla). The second sensillum type also contained two neurons, one that responded to Z9-14:Ac (1.3 ± 0.7 spikes per s spontaneous activity, 4 of 21 sensilla). The other neuron in this sensillum was unresponsive to any of the chemicals tested (0.3 ± 0.2 spikes per s spontaneous activity). Because of the small sample size, the actual distribution of different types is probably different from the 10:7:4 ratio in this study.

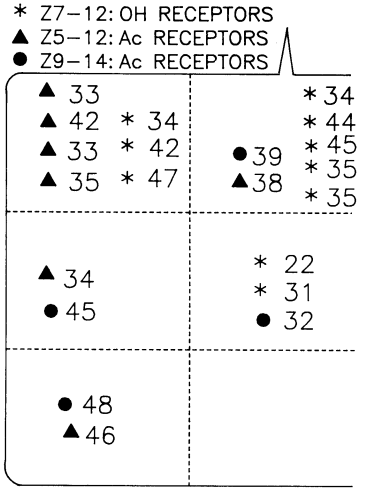

FIGURE 5 Template of a male *A. falcifera* antennal subsegment showing the distribution of recording sites for the three types of sensillum. The antennal flagellum has about 70 subsegments in total; for technical reasons most of the recordings were obtained between subsegments 30 and 45, counting from the base. Each subsegment was divided into 12 sections. The midline of the subsegment is indicated by the spike at the top of the template. The numbers indicate the subsegment number at which the recording was obtained, and the symbol indicates the sensillum type. Most of the sensilla with neurons sensitive to Z5-12:Ac were located along the edge of the subsegment. The other two types of sensillum appeared to be more evenly distributed.

B. Neurophysiologic and Behavioral Comparisons with Other Plusiinids

There are a number of similarities between the different sensillum types on these three Plusiinid moths. Each species has one sensillum type with a neuron that responds to the main pheromone component, Z7-12:Ac, and a neuron that responds to Z7-12:OH. In *T. ni* and the *P. includens*, a second type of sensillum contains two neurons of similar spontaneous activities and spike amplitudes, one that does not respond to any known pheromone component and a second that responds to either Z7-14:Ac or Z5-12:Ac. the *A. falcifera* antenna has two other types of sensillum, one that has neurons sensitive to Z7-12:Ac and Z5-12:Ac, found primarily along the outer margin of the subsegment (Figure 5). The second type is a rarely encountered sensillum with a neuron that responds to Z9-14:Ac. Rare types like the Z9-14:Ac chemoreceptor have been reported elsewhere as well (e.g., Van der Pers and Löfstedt, 1986).

Analysis of the behavioral responses of other Plusiinid moths to Z7-14:Ac, Z5-12:Ac, and Z9-14:Ac suggests that many such similarities occur in their pheromone receptor systems. *T. ni* is attracted to combinations of Z7-12:Ac with Z5-12:Ac and Z9-14:Ac, both of which inhibit the sexual response of *P. includens* to Z7-12:Ac (Table 1) (Figure 1) (Landolt and Heath, 1987; Linn et al., 1988). The closest relative of *T. ni, Trichoplusia oxygramma* (Geyer), is attracted to combinations of Z7-12:Ac with Z9-14:Ac (Landolt and Heath, 1986). Z9-14:Ac inhibits the response to Z7-12:Ac in *Autographa californica* (Speyer) and *A. flagellum* (Steck et al., 1979a). Z7-14:Ac inhibits the sexual response of *A. falcifera* (Steck et al., 1979b). It is not likely that these attractive or inhibitory effects occur unless receptor neurons that are sensitive to these chemicals at low stimulus intensities occur in at least small numbers on the male antennae of the respective species. One hesitates to predict from such a small sample, but it seems likely that at least a small number of receptor neurons for Z7-12:Ac, Z7-12:OH, Z5-12:Ac, Z9-14:Ac, and Z7-14:Ac will be found on the antennae of most other Plusiinid species. If so, the differences among Plusiinid pheromone chemoreceptor systems may lie primarily in the proportions of different sensillum type and how the neural responses are integrated in the central nervous system (CNS).

C. Inferences About the Evolution of Pheromone Receptor Neurons

Insect sex pheromone communication is a chain of three complex activities: (1) the production, storage, and release of a species-specific blend of pheromone components, (2) the detection and discrimination of the pheromone blend, and (3) the tracking of the pheromone plume to the potential mate.

Such a system is expected to be evolutionarily stable (Haynes et al., 1984; Mitter and Klun, 1987) because a mutation affecting any of these activities would survive in the population only if it maintained an unbroken chain of communication. Changes are expected primarily in an environment in which a mutation restores communication between conspecifics after it has been disrupted by a sudden introduction of conflicting or masking signals from other animals (Alexander, 1962; Lundberg and Löfstedt, 1987). Comparative studies of the pheromone components emitted by females of closely related Noctuid and Tortricid species are in agreement with such an expectation (Roelofs and Brown, 1982; Doré et al., 1986; Renou et al., 1988; Horak et al., 1988). Of 467 pheromones cited for female Noctuid moths, 68% include one of the four components: Z7-12:Ac, Z5-12:Ac, Z9-14:Ac, or Z11-16:Ac (Renou et al, 1988). The similarities among *T. ni, P. includens,* and *A. falcifera* pheromone chemoreceptors are also supportive of a hypothesis that evolution of the pheromone chemoreception system has been a slow process.

The evolutionary processes that may have occurred to create the differences that now exist among pheromone chemoreceptor systems are yet to be determined. A possible mechanism, similar to that proposed by Dethier (1980) for contact chemoreceptors specialized to detect particular secondary plant compounds, is that independent mutations first led to the presence of additional pheromone components and chemoreceptor types that had no initial role in the communication system. After the communication system was perturbed, a serendipitous mutation produced a change in the way input from different chemoreceptor types was integrated in the CNS. This permitted new behaviors to become associated with the previously disregarded stimuli.

Dethier proposed his original hypothesis on the basis of similarities and differences in the contact chemoreceptors of different lepidopterous larvae. The sensilla styloconica usually have three or four receptor neurons (e.g., Ma, 1972; Schoonhoven, 1972; Den Otter and Kahoro, 1983). Three of these neurons have similar specificities, for salts, water, and sucrose, respectively. In larvae of many moth species (Schoonhoven, 1973; Stadler, 1984; Frazier, 1986), the fourth styloconic neuron responds to secondary host plant substances. The specificity of this fourth neuron is considerably different in different species, even when the species exist on similar host plants (Den Otter and Kahoro, 1983). Dethier proposed that the prototype fourth neuron was a generalist. In those insects in which the fourth neuron became a deterrent receptor, an adaptive decrease occurred in the sensitivity of this neuron to compounds present in the host plant (Bernays and Chapman, 1987). Support for such a hypothesis comes from comparative studies of *Yponomeuta* chemoreceptors by Van Drongelen (1979). Similarly, in those insects in which the

fourth neuron became a stimulant receptor, an adaptive decrease occurred in the sensitivity of this neuron to compounds not present in the host plant. In both cases, the evolution would be driven by changes in oviposition behavior (Van Drongelen, 1979).

To consider how the hypothesis might apply at the chemoreceptor side of the pheromone communication system, first suppose that there are multiple alleles coding for the chemoreceptor sensitive to the major sex pheromone component of the ancestral Plusiinid prototype. One example of this condition is the gene coding for green-sensing pigment in human color vision (Nathans et al., 1986). It may also occur with the gene coding for a pheromone receptor in the red-banded leafroller moth (Chapman et al., 1978). Occasionally a mutation occurs at one of these alleles, and a small group of neurons appears on the antenna with specificity for a slightly different compound. The presence of this new receptor type is only slightly deleterious because of the high degree of convergence in the deutocerebrum (e.g., Christensen and Hildebrand, 1987). The sensitivity to a particular pheromone component in the CNS increases in proportion to the square root of the number of receptor neurons sensitive to it (Mankin and Mayer, 1983), so a small reduction in the number of neurons sensitive to the major components has very little effect on the overall CNS sensitivity. Over time, random mutations lead to a proliferation of receptor neuron types in the population. All the males have receptor neurons sensitive to the major pheromone component, and different individuals have small numbers of receptor neurons sensitive to compounds that differ slightly from the original major pheromone component. Occasionally, one of these mutations enhances the sensitivity to a minor component emitted solely by conspecific females or one emitted solely by females of other related species.

Once a pool of different individuals with receptor neurons sensitive to slightly different pheromone components develops in the population, selection may occur whenever a change in the environment affects the communicatión channel. Because the progeny from interspecific matings are usually less viable than from conspecific matings, strong selection pressure favors males whose central nervous systems discriminate in favor of blends emitted solely by conspecific females. Likewise, selection favors males that discriminate against blends emitted solely by females of other species. Subsequent mutations in regulatory genes controlling the quantity of receptor protein (as in Jan et al., 1987) may effect an increase in the percentage of receptor neurons sensitive to this component. Conversely, other mutations may reduce the number of neurons sensitive to a given component when the original selection pressures for discrimination are eliminated (e.g., Nagy and George, 1981; Löfstedt et al., 1986). Finally, all this occurs while the blends of pheromone components emitted by conspecific females and those of other females

in the environment undergo their own evolutionary changes. This results in an assemblage of several species that share one or more pheromone components in common and share a number of different types of pheromone chemoreceptor in common but discriminate for or against other components (or particular ratios of components) depending on the patterns of mutation of pheromone production of the local conspecific and interspecific females.

In applying such a hypothesis to the three insects in this report, it appears that pheromone production mutations in the ancestors of *T. ni* females led to the emission of Z7-14:Ac, Z9-14:Ac, and Z5-12:Ac in addition to the Z7-12:Ac emitted by the common ancestor of the three insects. Mutations ending the production of Z7-12:OH may have occurred in an ancestor of *T. ni* and *P. includens*, but not in *A. falcifera*. Chemoreceptors detecting these compounds either were present on antennae of ancestral males or evolved independently in all three species. The behavioral responses to the new components thereafter evolved differently for each species, depending on the pheromone blends emitted by conspecific females.

A critical concern in the hypothesis is how input from a new type of chemoreceptor becomes integrated into CNS decision processes. Are additional mutations required in the CNS before a new chemoreceptor can elicit a change in sexual behavior? How does the new input reach a "command center" where pheromone blends are discriminated? Morphologic and developmental studies of insect antennae suggest a possible answer. The sensilla may proliferate in a pattern that permits neurons of any new or ancestral chemoreceptor type to converge upon separate loci in the CNS. On some insect antennae, sensilla of different types lie approximately in rows along the longitudinal axis of the subsegment (Steinbrecht, 1970; Sanes and Hildebrand, 1976), which may be the case for the Z5-12:Ac sensillum of *A. falcifera* (Figure 5). In others, the sensilla lie in rows along the circumference of the subsegment (Van Der Pers et al., 1980) or in small bundles or patches (Esslen and Kaissling, 1976; Ramaswamy and Gupta, 1981; Hansson et al., 1986). In developmental studies, Schneiderman et al. (1986) prepared gynandromorphic female *Manduca sexta* by transplanting antennal imaginal disks from male to female fifth instar larvae. The male pheromone receptor neurons converged normally in the female deutocerebrum, and behavioral responses were observed both to sex pheromones and to ovipositional attractants. These patterns of neural distribution conform to a developmental scheme that was proposed from genetic studies of *Drosophila* homoeotic mutants (Campos-Ortega and Hartenstein, 1985). In this scheme, the position of the sensillum maps to a distinct territory in the CNS. For pheromone receptor neurons, this territory is probably a locus in the macroglomerulus of the deutocerebrum (Christensen and Hildebrand, 1987). The termination sites in the CNS seem to be organized according to olfactory specificity (Stocker et al., 1983) in a

segmentally repetitive fashion (Campos-Ortega and Hartenstein, 1985; Rospars, 1988). Thus, the axons from patches or rows of sensilla located at approximately the same position on each subsegment are expected to terminate at the same locus in the CNS. Because the small number of neurons on each subsegment would map together at the same locus in the CNS, this could enable individual receptor neuron responses to be integrated together for greatest effect (Light, 1986). Koontz and Schneider (1987) detected such a pattern in the macroglomerulus of *Bombyx mori*. This pattern of convergence would be particularly important when there are only a few receptors of a specific chemoreceptor type on the antenna.

Less is known about the ultimate fate of the signals from new chemosensory types that converge at a command center in the CNS. It seems plausible, however, that the command center undergoes a process of "coevolution" under separate genetic control and that selection determines whether the new input (or even older input) is excitatory, inhibitory, or neutral. In *Ostrinia nubilalis*, for example, an allele for different pheromone chemoreceptor phenotypes appears to be autosomal and independent of a sex-linked allele for different types of behavioral response (Roelofs et al., 1987).

IV. SUMMARY

Insects sense their chemical environment through receptor neurons inside sensilla on the antennae, mouthparts, and legs. Some of these neurons detect a broad spectrum of chemicals, but others are highly selective for specific chemicals used as mate or host identification cues. A relatively unexplored question about such chemoreceptors is the coevolution of receiver and sender. How do chemoreceptors evolve in correspondence with evolutionary changes in the production of their key stimuli?

Two receptor systems for which coevolution is of particular interest are the pheromone chemoreceptor system and the larval contact chemoreceptor system. Pheromone chemoreceptors are selective for sexually excitatory odorants (sex pheromones) emitted by conspecifics or for sexually inhibitory odorants emitted by individuals of closely related species. Some contact chemoreceptor (taste) neurons on larval mouthparts are selective for secondary chemicals present in (or absent from) host plants. Much of what is known now about the evolution of chemoreceptor specificity comes from studies of contact chemoreceptors, but the two systems are similar in many respects.

To consider the evolution of specificity in pheromone chemoreceptors, responses from neurons on the antennae of the celery looper moth (*Anagrapha falcifera*) were recorded to six pheromone components tested in previous surveys of two close relatives, the soybean looper (*Pseudoplusia includens*) and the cabbage looper (*Trichoplusia ni*). It was found that these three moths

share in common one type of sensillum with two pheromone-sensitive neurons of the same specificity. This sensillum type may have passed unchanged from a Plusiinid ancestor, in which case it may be found on the antennae of most Plusiinid species. The antennae also contain other pheromone-sensitive receptor neurons of apparently unique specificities. The evolution of neurons with unique pheromal specificities was discussed in this chapter in reference to previous hypotheses about the coevolution of plants and insect herbivore contact chemoreceptors.

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