Spatial Arrangement of Different Types of Pheromone-Sensitive Sensilla in a Male Moth

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Male moths perceive the female-emitted sex pheromone components by thousands of antennal sensilla of the same general morphology [1]. Different physiological types of pheromone-receptive sensilla, each with receptor cells responding to a specific component, have been distinguished [2, 3]. We have found that in the Heart and Dart moth *Agrotis exclamationis* (L.) (Lepidoptera: Noctuidae), the pheromone components are detected by two different receptor cells in different sensilla, which differ in their morphology and in their spatial localization on the antenna. The spatial arrangement of receptor cells on the male antenna might be an adaptation to optimize the detection of the minor component in the female pheromone.

The antenna of male *A. exclamationis* consists of 80–90 segments with a total length of about 10 mm. It has no lateral branches, and the basal and medial 2/3 of the length (about 40 segments) has a relatively large diameter (250–300 µm) compared with the distal filiform part (150–250 µm). Two morphological types of single-walled sensilla [1] dominate on the basal and medial part of the antenna. The most conspicuous type forms dense bundles of each about 20 sensilla at the lateral margins of all the basal and medial antennal segments (Fig. 1 A). These sensilla are slightly curved and have a length of about 200 µm, and a diameter of 4–5 µm at the base. More central to these there is a second type of sensilla which has a gradually decreasing length, the shortest towards the midline with a length of about 100 µm and a basal diameter of 3–4 µm.

In the transmission electron microscope, another feature emerges which further emphasizes the difference in morphology between the lateral and medial sensilla besides their length and diameter; the sensilla in the marginal bundles are innervated by a single sensory cell, whereas those closer to the midline are innervated by two sensory cells (Fig. 1 B). The transition zone between the two morphological types may be sharply defined, or in some cases with a certain intermingling of the two types. Unbranched sensory processes emerge from the neurons and extend to the distal part of the hair in both kinds of sensilla. The number of pores in the hair wall is moderate and without any striking difference between the two kinds of sensilla.

The two main pheromone components in *A. exclamationis* are (*Z*)-5-tetradecenyl acetate (*Z*5–14:OAc) and (*Z*)-9-tetradecenyl acetate (*Z*9–14:OAc). Bestmann et al. [4] found these compounds in a 93:7 ratio in females matching a maximal trap catch of males at a ratio of 95:5. Vrkoc et al. [5] reported that addition of the dodecenyl and hexadecenyl acetate fractions from the female increased the behavioral activity of the tetradecenyl acetates, but no additional compounds could be identified. Using single-cell techniques, Priesner [6] found specific receptors on the male antenna for the two main components mentioned above, but also for three other compounds: (*Z*)-7-dodecenyl acetate (*Z*7–12:OAc), (*Z*)-7-tetradecenyl acetate (*Z*7–14:OAc), and (*Z*)-11-tetradecenyl acetate (*Z*11–14:OAc).

In a first experiment we obtained identical electroantennogram dose-response curves for *Z*5- and *Z*9–14:OAc. The threshold value was 1 µg loaded on the filter paper in the stimulus syringe. We then measured the electrophysiological response of 50 lateral and 50 medial sensilla, with the tip-recording technique [7]. Their responses to the following compounds (10 µg applied on filter paper source), were recorded: *Z*5–10:OAc, *Z*3–12:OAc, *Z*5–12:OAc, *Z*7–12:OAc, *Z*5–14:OAc, *Z*7–14:OAc, *Z*9–14:OAc, *Z*11–14:OAc, *Z*7–16:OAc, *Z*11–16:OAc, *Z*5–14:OH, and *Z*9–14:OH. Among the lateral sensilla three physiological types were found: the most

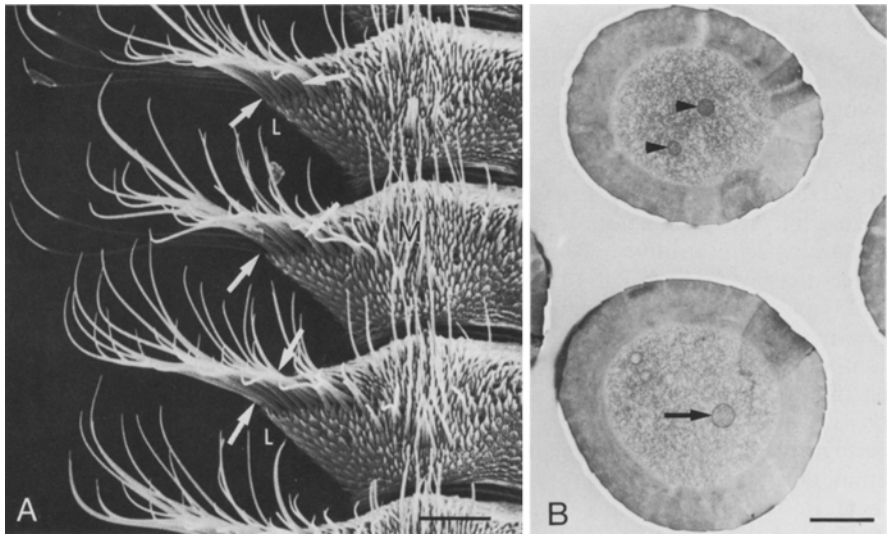

Fig. 1. A) Scanning electron micrograph showing the middle part of a male antenna of *A. exclamationis*. The sensilla occur on the ventral side of the antenna. The long, marginally placed pheromone-sensitive sensilla are present in bundles of about 20 (*between arrows*) on the lateral edge (*L*) of the basal and medial antennal segments. Towards the midline of the antenna (*M*) the sensilla gradually decrease in length. Scale bar 50 µm. B) In the lateral bundle the pheromone-sensitive sensilla have one sensory cell (*arrow*), whereas the sensilla towards the midline have two sensory cells (*arrowheads*). In this transmission electron micrograph two neighboring sensilla from the transition zone are shown in cross-section through their basal parts. Scale bar 1 µm. The specimens used in the electron microscopic studies were processed according to standard methods [10]

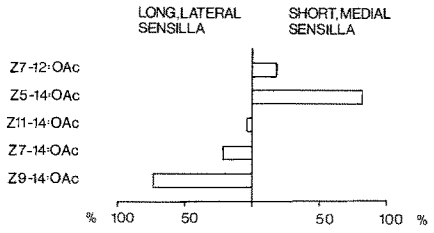

Fig. 2. Frequency of receptor cells in lateral (long) and medial (short) sensilla on antennae of male *A. exclamationis* specifically responding to pheromone components and three related chemicals ($n = 50$ for each sensillum type). For experimental details see text

common one responded mainly to *Z*9–14:OAc, a second type to *Z*7–14:OAc with a weak response also to *Z*9–14:OAc, and a third type responded exclusively to *Z*11–14:OAc (Fig. 2).

The medial shorter sensilla towards the antennal axis have two cells: Cell A with a high spike amplitude and cell B with a low spike amplitude. The A cell responds to totally different pheromone components than the cells in the marginal long sensilla: the majority (82%) responds to *Z*5–14:OAc, whereas a lower number (18%) is activated exclusively by *Z*7–12:OAc (Fig. 2). The function of the B cell is still unknown. It did not respond to any of the compounds tested. These results were corroborated by measuring the response of ten short sensilla to the same compounds with the penetration technique [8].

The receptor types found by us are thus in agreement with what Priesner reported [6]. The proportion of sensilla-containing cells sensitive to compounds other than *Z*5- and *Z*9–14:OAc is striking. Trapping experiments by Priesner [6] showed that these other compounds have an inhibitory effect on the attraction of male *A. exclamationis*. Our own unpublished gas chromatographic analyses on capillary columns did not reveal traces of any of them in female extracts (<0.5% of *Z*5–14:OAc). They are, thus, most probably pheromone compounds in some other competing species. More interestingly, however, we found a clearcut correlation between the morphology of individual sensilla, their spatial localization, and their sensitivity to different compounds. The receptors for the pheromone component *Z*9–14:OAc are exclusively arranged along the lateral margins of the antennal segments, and are each innervated by one neuron. The *Z*5–14:OAc receptors are all placed medially in sensilla containing also a second cell. Such a spatial arrangement of receptors sensitive to different pheromone components has not been reported before.

It is interesting to note that *Z*9–14:OAc is perceived by a specific cell in the long distal sensilla placed on the edge of the male *A. exclamationis* antennal segments. There is in the pheromone blend an optimal proportion of this component to *Z*5–14:OAc of less than 10% for male attraction. The proportion of molecules perceived by the sensilla from the air is by aerodynamic laws much higher for distal sensilla than for central ones [9]. This means that the localization of the *Z*9–14:OAc receptors on the male *A. exclamationis* antenna could be adaptive for sensitive detection of the minor one of the two pheromone components.

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Dosage Response to Ethanol Mediates Host Selection by "Secondary" Bark Beetles

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We report a unique dosage response pattern in which ethanol affects aggregation of scolytids (Coleoptera: Scolytidae) on host trees: High ethanol concentrations enhance the response of nonaggressive ("secondary") bark beetles and ambrosia beetles, but interfere with the pheromone response among aggressive species. Species depending on fresh host material but unable to infest live trees show an intermediate response pattern: a low ethanol concentration increases, high concentration reduces attraction to host odors and/or pheromonal compounds.

Ethanol generates naturally by anaerobic fermentation in the moist phloem and sapwood of logs, stumps or diseased trees [1], presenting temporary habitats for numerous insects. Conflicting reports on the role of ethanol in bark beetle aggregation and/or host colonization [2, 14, 17] led us to investigate the effects of ethanol concentrations on the response of four bark beetles (*Ips typographus* [L.], *Leperisinus varius* [F.], *Hylurgops palliatus* [Gyll.], *Tomicus piniperda* [L.]) and four ambrosia beetles (*Trypodendron lineatum* [Oliv.], *Xyleborus dispar* [F.], *Xyleborus saxeseni* [Ratz.], *Xylosandrus germanus* [Blandf.]) to traps baited with their respective pheromones and/or ethanol. Included was also a woodboring lymexilonid, *Hylecoetus dermestoides* L.

Field tests were conduced near Freiburg/Br. using commercial flight barrier traps (Röchling, Haren/Ems, FRG) spaced approx. 15 m apart in latin square design. Ethanol of different concentrations was eluted from 0.05 mm low-density polyethylene bag dispensers (7 × 10 cm) or glass vials of different size to give a wide range of release rates (0.5–250 mg h^{-1} as estimated in the laboratory resp. 0.1–0.001 mg h^{-1} by using tenfold di-