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STRUCTURE AND FUNCTION OF OLFACTORY SENSILLA ON THE ANTENNAE OF SOYBEAN APHIDS, *APHIS GLYCINES*

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Abstract by authors: Observation were made on the morphology of antennal sensilla of *Aphis glycines* using scanning electron microscopy. Apterae have antennal sensilla similar to those of the alatae, A group of four stout and often blunt-ended hairs appear at the tip of the antenna. A flattened sense organ(primary rhinarium) is located on the fifth antennal segment and that on the sixth segment there are four sensilla coeloconica and two sensilla placodea. They are ringed with a fringe of cuticle of which the finger-like extensions might function as a protective sieve against the entry of undesirable particles. Secondary rhinaria consist of sensilla placoidea resembling that on the fifth antennal segment, but without the elaborate fringe. Alatae differ even more markedly from apterae by possession of several to many secondary rhinaria on the flagellum. There were many secondary rhinaria on the third and the fourth, even the fifth segment in male *A. glycines*.

The olfactory site of tested chemicals were analyzed by making use of EAG technique. It is demonstrated that primary rhinarium on the sixth antennal segment in slate Virginoparae responds to terpene derivatives(their alcohols, a1dehydes and esters), but not to terpene hydrocarbons, While the rhinarium on the fifth segment responds to terpene hydrocarbons and not to terpene derivatives. Green leaf volatile and aromatic compounds were perceived by primary rhinaria on both segments, but the intensities of olfactory responses to the chemicals in each rhinarium are different. Besides the primary rhinaria on the two segments, receptor cells which responded strongly to (E)-2-hexenal and 1-hexanal were found on other sensilla, which might be the trichodea (1μm)and the small placodea (0.8μm) on the fifth segment. However, there was no direct electrophysiological evidence for it. Primary rhinarium on the sixth segment consists of main olfactory receptors for 3-octen-1-ol; moreover, trichodeum and small placodeum on the some segment might also contribute to the sensory response to 3-octen-1-ol. Primary rhinarium on the sixth segment was proved to be the sensory site for (E)-ß-farnesene in alate and apterous virgenoparae. Caryophyllene, which is an inhibitor of alarming pheromone, and (E)-ß-farnesene tarnesene could evoke a weak response in the secondary rhinaria on the third segment, and the primary rhinarium in apterous virginoparae.

Key words *Aphis glycines*, olfactory sensillum, electroantennogram, olfactory site, aphid pheromone, plant volatiles

The microstructures of the antennal sensilla of some aphids have been extensively reported [1-5]. However, this research merely describes morphology and structure. Early work employed the method of cutting off sensilla to study behavioral responses of aphis to alarm pheromone [6] and sex pheromone [7, 8] from behavioral standpoint. Bromley *et al.* [9] used single sensilla to record and determine the different olfactory responses of the primary rhinarium, located on the fifth antennal segment and the sixth antennal segment of *Nasonovia ribisnigr,i* to more than 70 kinds of plant volatile secondary materials for the first time. Dawson *et al.* [12] used a single cell recording method to record the olfactory responses of the secondary rhinarium, located on the antennae of *Megoura viciae* and *Schizaphis graminum*, to sex pheromone nepetalactol and nepetalactone. Dawson *et al.* [10][11] also used single cell to record the olfactory responses of the primary rhinarium, located on the *Lipaphis erysimi*, to alarm pheromone. This work helped to familiarize us with the functions of aphis antennal sensilla. However, a review of previous research on a few aphis antennal sensillas and their functions shows that we are still short of a comprehensive understanding of them. We have studied the olfactory responses of the *Aphis glycines* to plant odors and the composition of its host odor (to be published in future). In order to get to know the olfactory feeling characteristics of *A. glycines*, and advance the exploration of aphis olfaction mechanisms, this paper reports the microstructures and their functions of the antennal sensillas for each kind of *A. glycines* in detail.

1. Material and methods

1. 1 Experimental insects

A. glycines were captured from *Rhamnus davurica* near Qinghe in Beijing's suburban area in spring, and were fed on indoor *Glycine max* plants (dumpy grass, Shangyu, Zhejiang) under room temperature and illumination conditions.

1. 2 Chemical reagent

1. 2. 1 The samples of plant volatile and secondary compounds were bought from two companies, Roth and Fluka. The purity was 95%-99%. (E)-ß-farnesene (EBF) was provided by Professor Zhongning Zhang from the Institute of Zoology, Chinese Academy of Science.

1. 2. 2 The Kaissling solution reagents with analytically pure levels used in the electrophysiological experiment were all produced by Beijing Chemical Reagent Plant.

1. 3 Observation using electron microscopes

1. 3. 1 Specimen-making process: aphids were fixed in 3% glutaraldehyde for 24

hours, rinsed three times in phosphate buffer-liquor (pH 7.2), then processed in electrically conductive liquor, and washed in heavy distilled water. After naturally drying, aurum was sprayed.

1. 3. 2 The processed aphids were observed using a Hitachi H-3010 scanning electron microscope. The accelerating voltage was 20kV.

1. 4 Antennae potential record

The anterior body of the aphid was cut off between the prothorax, and the middle metathorax was cut off before the test; one fore leg and one antenna were also cut off. The top of another antenna was cut off for spare use. The reference electrode was inserted into the base of the antennae, and the recording electrode sheath was on the top of the antennae. A vitreous electrode was drawn and formed using vertical capillary drawing-ware (KOPF 720). The inside diameter of the capillary was 2mm. A proper amount of Kaissling solution was filled into the vitreous electrode. The prescription or composition of the solution was Glucose (354mmol/L), calcium chloride (1mmol/L), potassium dihydrogen phosphate (20mmol/L), potassium chloride (64mmol/L), magnesium chloride (12mmol/L), sodium chloride (12mmol/L) and potassium hydroxide (9.6mmol/L), with the pH value of 6.5[13]. The silver - silver chloride electrode with a diameter of 0.2mm was placed into the glass electrode, and the glass electrode connected the microelectrode alternating/direct-current amplifier (Nihon, Kohden, MEZ-7101), postpositional amplifier (Nanjing electrophysiological apparatus plant, FzG-1A), oscillograph (Hameg, HM-203-6) and recorder (Gould, Recorder 220) [13,14].

In order to decrease the volatilization of the chemical material, each reagent was dissolved in paraffin oil. No forms of A.glycines responded to paraffin oil. The concentration of all test materials was 1% (v/v) except the concentration dosage reaction, which was much higher than the sensation threshold of *A. glycines* to plant odor (as will be published in future, for 10^{-4} or 10^{-5} volume ratio concentration). 25μl of mixed solution was absorbed and uniformly dropped onto 6x0.5cm^2 filter paper each time, and put it into a burette. The terminal of the burette connected the control equipment activated by gas, the top inserted into pinholes in the glass tube arrangement with successive pneumatic flow. The flux of successive pneumatic flow was 80ml/min and stimulated the antennae of *A. glycines*. The antennal stimulation time for each attempt was 0.2 second, and the time interval between two stimulations was more than 30 seconds. This experiment was repeated six times (to test six antennae).

2. Results and analysis

2. 1 Morphological structure

The electron microscope (SEM) observation showed that the antennae of *A. glycines* were similar to those of many other aphids. The primary rhinarium is distributed on the fifth and sixth segment. The primary sensillum distributed on the fifth segment was a big sensillum placodeum. The primary rhinarium distributed on the sixth segment consisted of two sensillum placodeums and four s. coeloconicums. The primary rhinarium existed in all forms and adult and nymphs of *A. glycines* (plate I: 1,3). They are ringed with a fringe of cuticle that formed the finger-like extensions, which might function as a protective sieve against the entry of undesirable particles.

Alate aphids have the secondary rhinarium on the third segment (plate I:5). This is different from the apterous parthenogenesis aphid and its nymph, the latter one lacking such secondary rhinarium. The alate sexupara aphid in autumn has more secondary rhinarium than alate parthenogenetic aphids. A diameters of these seconday sensilla are around 13μm. The alate male aphid has more seconday sensilla, on the fourth and fifth segments (plate I:6), distributed either individually or in pairs. The seconday sensilla are also sensillum placodeums, which are similar with the primary rhinarium on the fifth segment, but they lack the fine fringe of the cuticle.

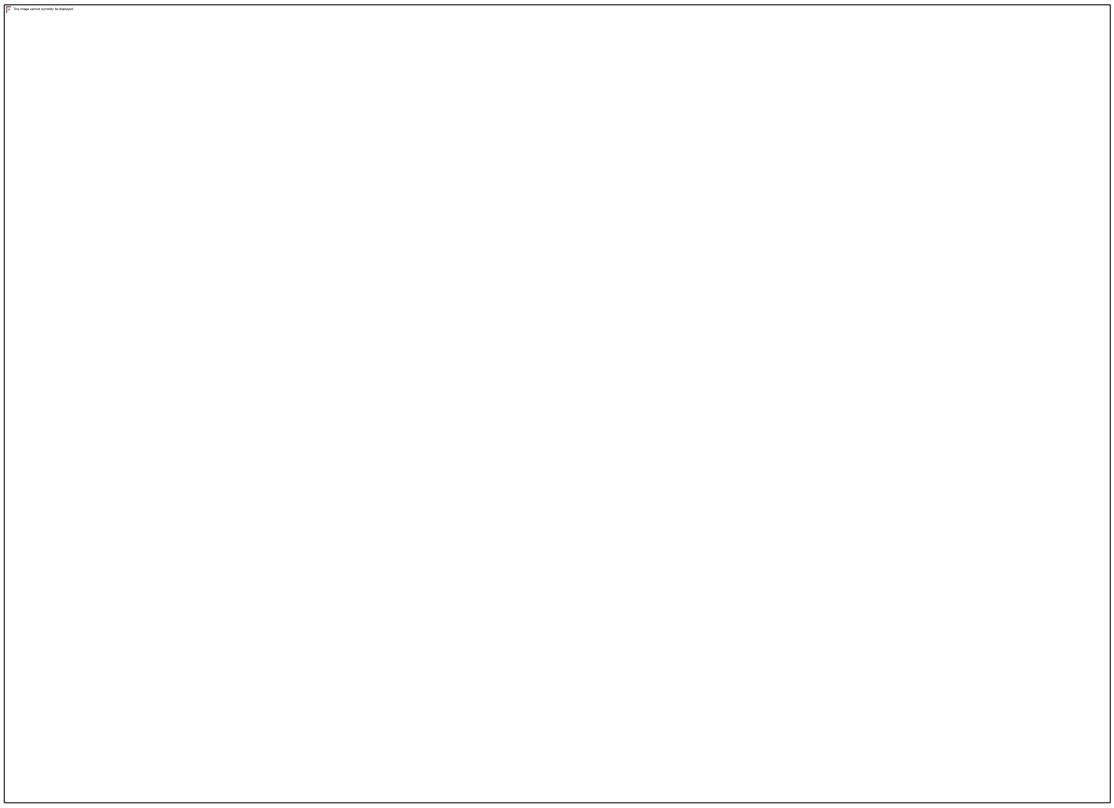
A group of four stout and often blunt-ended hairs appear at the tip of the antenna of *A. glycines*. A diameter of each hair is 0.83μm; and the length is about 1μm. When *A. glycines* walk on the plant cuticle, they often move their antennae forward and downward. The s. trichodeums often touch the plant cuticle. The electrophysiological record shows that these s. trichodeums play an olfactory role in the process of *A. glycines'* host selection, which could be used to perceive the chemical composition of the plant cuticle. It has been also reported that antennae movement is also related to the needling activity of *A. glycines*.

2. 2 The function of *A. glycines'* olfactory sensilla

2. 2. 1 Alarm pheromone sensory sites

The response EAG values of the alata parthenogenesis aphid, apterous parthenogenesis aphid and alata 4 instar aphid to (E)-ß-farnesene are shown in Fig.1.

It can be seen from the figure that the response of the alata parthenogenesis aphid and apterous parthenogenesis aphid of *A. glycines* has little difference to the EAG values of (E)-ß-farnesene. In addition, there is also little difference between the alate 4th instar aphid and the fully-grown aphid. But the response of the alate 4th instar aphid is relatively low to the EAG values of c-3-octen-1-ol, only 0.087±0.032mV(n=6), which is very different from the adult aphid. The measured value of the latter was 0.215±0.054mV(n=6). Therefore, we infer from the comparison of these data that relatively single olfactory receptors exist in each adult of *A. glycines* and its nymph to the alarming pheromone.



When the capillary head that was used to record the electrode covered the primary rhinarium on the sixth segment, the EAG response values of the alate parthenogenetic aphid to EBF decrease by 74.4±10.9 (EBF concentration was 10^{-1}V/V) and 88.8±10.7 (10^{-2}V/V). However, the EAG response values of the apterous parthenogenesis aphid to EBF decreased by 88.8±10.7 (EBF concentration was 10^{-1}V/V) and 96.8±4.6 (10^{-2}V/V). As for alate parthenogenetic aphid, the primary rhinarium on the fifth segment is covered. However, the entire fifth segment and fourth segment do not hide the rest of the other rhinaria. Only when the secondary rhinarium on the third segment was completely covered, its EAG response vanished completely. But as for the alate parthenogenetic aphid, the EAG response vanished completely when the primary rhinarium on the fifth segment was covered.

We could conclude from the above results that the primary rhinarium on the sixth segment was the most important olfactory receptors site to (E)-ß-farnesene, and that the secondary rhinarium on the third segment of the alate parthenogenetic aphid has a certain perceptibly to EBF. The primary rhinarium on the fifth segment of the apterous parthenogenesis aphid has weak perceptibility to EBF. Furthermore, the above data also shows that the secondary rhinarium on the third segment and the primary rhinarium on the fifth segment respond more strongly to EBF with high concentrations. So it could be inferred that the sensory thresholds of some sense cells are relatively high.

In comparing the olfactory response differences of alate 4th instar nymphs and adult aphids to EBF, the response difference of these two aphids to EBF is due to the lack

of the secondary rhinarium in the third segment of the alate 4th instar nymph. Thus this also showed that the response olfactory receptor of the alate 4th instar nymph to alarm pheromone has obvious differences from the olfactory response sensitivity of the adult aphid. But the response olfactory receptor to the plant volatile secondary compound has obvious differences from the olfactory response sensitivity of the adult aphid.

2. 2. 2 The effecting sites to the plant volatile secondary compound

When the capillary head that was used to record the electrode covered the primary rhinarium on the sixth segment, the EAG response values to EBF of the following compounds decreased to zero: linalool, orange alcohol, geraniol, farnesene acetate, α-pentanone, (±)-ß-citronellol, lina-ester and terpineol. This showed that the primary rhinarium on the sixth segment is the olfactory receptor site to these compounds. Meanwhile, the EAG response of other several kinds of compounds did not change, which include myrcene, ß-pinene, α -pinene, R(+)-limonene and (+)-carane terpene. In addition, when the capillary head that was used to record the electrode covered the primary rhinarium on the fifth segment, the EAG response values to EBF of these compounds also decreased to zero. Obviously, the olfactory receptor sites of these compounds are on the primary rhinariums on the fifth and sixth segment.

It can be concluded from the above experiment that the olfactory receptor sites of *A. glycines'* sense terpene onium compounds exist on the primary rhinariums on the fifth segment and the sixth segment.

Table 1. The terpene compound perceived by the primary rhinariums on the fifth segment and the sixth segment of the alate parthenogenetic *A. glycines*

Site of the primary rhinariums	The fifth segment	The sixth segment
Compound	myrcene, ß-pinene, α -pinene, R(+)-limonene, (+)-glycoside terpene	linalool, orange turn alcohol, geraniol, farnesene acetate, α -pentanone, (±)-ß-citronellol, lina-ester and terpineol

In addition to the above compounds, some other compounds among the 39 kinds of compounds tested could be perceived by the primary rhinariums on both the fifth segment and the sixth segment. These compounds are some saturated and unsaturated alcohol, aldehyde, ester and their derivatives, which consist of six or seven carbon atoms; namely greenery odor and aromatic compounds (see Table 2).

Table 2. The olfaction response degree percentage of the primary rhinariums on the fifth segment and the sixth segment of the alata parthenogenesis aphid of *A. glycines* to greenery smell and aromatic compound (repeat for 6 times)

Greenery odor and aromatic	The olfaction response degree percentage

compound	The primary rhinariums on the fifth segment	The primary rhinariums on the sixth segment
1-pentanol	52. 5 ± 5.0	47.5 ± 5.0
N-hexyl alcohol	47.8 ± 6.7	52.5 ± 6.7
N-heptyl alcohol	40.3 ± 10.2	59.7 ± 10.2
1-nonanol	34.4 ± 3.3	65.6 ± 3.3
Antak	13.1 ± 10.1	86.9 ± 10.1
c-2-hexylene-1-alcohol	79.9 ± 10.3	20.1 ± 10.3
t- 2-hexylene-1-alcohol	68.0 ± 5.8	32.0 ± 5.8
c-3-hexylene-1-alcohol	47.5 ± 11.7	52.5 ± 11.7
t- 3-hexylene-1-alcohol	61.3 ± 14.5	38.7 ± 14.5
Benzaldehyde	56.8 ± 7.4	43.2 ± 7.4
Phenylacetic acid ester	48.1 ± 2.2	51.9 ± 2.2
c-3-hexylene acetate	75. 8 ± 22.1	24.2 ± 22.1
2-phenylethyl acetate	34.3 ± 5.8	65.7 ± 5.8

As for the compound 1-octylene-3-alcohol, the EAG response values decreased by 38 ± 19.3 when the primary rhinariums on the sixth segment are covered. But the EAG response vanished when the entire sixth segment was covered. This shows that there exist other rhinariums that have olfactory identification effects on 1-octylene-3-alcohol besides the primary rhinariums. The electron microscope (SEM) observation showed that only some hair-like trichodeums with a diameter of about 0.7μm and one small placodeum with a diameter of about 0.61μm were found besides the primary rhinariums on the sixth segement (plate I:1). However, there was no direct electrophysiological evidence for whether they might also contribute to the sensory response to 3-octen-1-ol or not.

As for the hexanal and (E)-2-hexenoic aldehyde, the EAG response values decreased by only 20% and 30% when the primary rhinariums on the sixth segment and the fifth segment were covered. The EAG response just vanished until the 1/3 of the fifth segment near the base was covered. This shows that there still exist other chemical rhinariums located from the 1/3 of the fifth segment near the base to the primary rhinariums that perceive hexanal and (E)-2-hexenoic aldehyde besides the primary rhinariums. But the electron microscope (SEM) observation showed that only one hair-like trichodeum (a diameter is about 1μm) and one small and single placodeum (a diameter is about 0.8μm) were found (plate I:4); no other rhinarium was found. These chemical rhinariums might perceive these two compounds. However, there was no direct electrophysiological evidence for it in terms of the current conditions.

On the other hand, the EAG response recovery time of these rhinariums to the hexanal, (E)-2-hexenoic aldehyde and 1-octylene-3-alcoholic was also found to be very long in the experiment. So this shows that the affinity between these specific compounds and olfaction receptors is very strong.

It could be seen from the concentration dose versus response curve that the curve of linalool is similar with those of geraniol, c-2-octen-1-ol, t-2-octen-1-ol, 1-hexanol, 1-heptanol and benzene ethyl ester. This is consistent with the content of this paper, namely they have similar olfaction receptor mechanisms respectively. But 1-hexanal and (E)-2-hexenal aldehyde are different from them.

The effecting site of (-)-(E)-pink terpene on *A. glycines* antennae lies on the third segment, which is an inhibitor of (E)-ß-farnesene tarnesene[11]. Part of the effecting site of EBF is also on the third segment.

But the olfactory response of every olfactory rhinarium of the apterous parthenogenetic aphid to the plant volatile secondary compounds has obvious differences from that of the alate parthenogenetic aphid. The primary rhinariums on the fifth segment of the apterous parthenogenesis aphid also respond to some compounds such as geraniol, orange alcohol and linalool. The rhinariums near the base of the fifth segment also somehow respond to 1-hexanal.

3. Discussions

The sensory system of aphids is not complex as compared among growth stages with similar individual sizes. Specialization during evolution omits all the sensillas except the most basic sensilla, and only leaves aphids with a limited sensory structures to meet common needs such as vision, flying regulation and control, taste, touch and olfaction [15]. The results of this experiment also show that the location of the olfactory sensilla on the *A. glycines* antennae is helpful in perceiving the odor stimulation of the outside environment. Meanwhile, the diverse functionality of the olfactory sensilla is also very clear, which could ensure that every form of aphid uses the olfactory scent to distinguish host plants from non-host plants.

The secondary rhinariums on the third segment of the alata parthenogenesis aphid doe not respond olfactorily to greenery odor and most of the terpene compounds. Among all the tested compounds, only (-)-(E)-pink-terpene responds to it. Therefore, the authors infer that it has no use in long-distance olfactory directing of *A. glycines* to host plants. In addition, it can be inferred if we compare the olfactory sensilla of every morphological form of *A. glycines* and its biology, many of the secondary rhinariums on the third, fourth and fifth segments of the male aphids are used to perceive the sexual pheromone excreted by female aphids.

Although we chose the EAG technique to do our analysis, it has some disadvantages and it is difficult to provide further information. In the ongoing research, single-cell-recording (SCR) technique would be used to analyze the perceiving response of each receptor cell to each

compound. Thus, this research work will build a good foundation for future research on the olfactory coding of the *A. glycines* to aphid pheromone and plant odor.

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