Female Sex Pheromone Glands in the Parasitic Wasps,
Genus *Apanteles*¹

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(Received March 10, 1983)

To locate female sex pheromone glands of the parasitic wasps *Apanteles*, 7 species of this genus were investigated, which were *A. plutellae*, *A. lippardii*, *A. baoris*, *A. kariryi*, *A. ruficus*, *A. sp* (1), and *A. sp* (2). Behavioural tests on *A. plutellae* showed that most of the males (13/20) responded to the ovipositor and the 9th tergite of the female with vibrating their wings, and that 4 and 3 of 11 males showed wing vibrating behaviour to the ovipositor and to the 9th tergite respectively. Histological observations on every *Apanteles* species manifested that a pair of secretory glands located on the inner surface of the 2nd valvifer and that a pair of patches of epithelial secretory cells on the 9th tergite. The organs on the 9th tergite, however, were also found in males. Therefore, the glands on the 2nd valvifers were considered the sex pheromone organs. SEM observations on the outer surfaces of the female genitalia of some *Apanteles* species supported this conclusion, because there were some tiny pores on the 2nd valvifers corresponding with the loci of the glands and no pores on the 9th tergites. Sex pheromones of *Apanteles* group were considered to be secreted through these pores.

**INTRODUCTION**

There have been many reports on the existence of female sex pheromones in the Hymenoptera (Murr, 1930; Whiting, 1932; Bousch and Bærwald, 1967; King et al., 1969; Cole, 1970; Vinson, 1972, 1978; Assem and Povel, 1973; Matthews, 1974; Obara and Kitano, 1974; Weseloh, 1976; Yoshida, 1978).

In some species, the pheromone organs were determined. Mertins and Coppel (1972) described in a pine saw-fly *Diprion similis*, a pair of possible sex pheromone glands located at the anterolateral margins between the 2nd and the 3rd abdominal segments. In the honey bee *Apis mellifera*, Gary (1962) reported that the mandibular glands of the queen had a role in a sex pheromone secretion.

In parasitic Hymenoptera, few works have been done on the localization of pheromone producing organs. In the ichneumonid wasp *Campoletis sonorensis*, Vinson (1972) reported that all the female parts elicited male's mating behaviour, and suggested that the pheromone was secreted through the cuticle. In the braconid wasp *Cardiochiles nigriceps*, Vinson (1978) presented the possibility that a DuFOUR's gland was the

¹ This work was supported in part by the Grant-in-Aid for Special Research Project on the Mechanisms of Animal Behaviour from the Japanese Ministry of Education, Science and Culture.

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source of a sex pheromone. In another braconid wasp *Apanteles glomeratus*, Tagawa (1977) reported that the female sex pheromone gland was located at the base of the 2nd valvifer which was a component of the female genitalia. However, in a solitary parasitoid *Apanteles melanoscelus*, Welshol (1980) reported that the source of the pheromone was on the 9th tergite (his 8th tergite: morphologically, his 8th tergite should be regarded as the 9th tergite) which had a pair of secretory glands. If this is the case, *Apanteles* group is interesting, for the different species in the same genus have different types of female sex pheromone organs. It is therefore important to examine other *Apanteles* species comparatively.

Present work was then carried out to find out female sex pheromone glands in some *Apanteles* species collected in Japan.

**MATERIALS AND METHODS**

Seven *Apanteles* species were investigated, which were *A. plutellae*, *A. lipardis*, *A. baoris*, *A. ruficrus*, *A. kariyai*, *A. sp* (1), and *A. sp* (2). Among them, *A. plutellae* is a solitary parasitoid, and the others were lizards. All of them were larval parasitoids of lepidopteran hosts. Host species of these parasitoids were listed in Table 1.

The parasitoids were obtained from the parasitized host larvae collected in the field from 1978 to 1982. Collected host larvae of each parasitoid species were reared on their own food plants respectively at room temperature. The parasitoids after egression were also maintained at room temperature. Adult wasps were fed on honey drops and water.

Wasps within a week after emergence were used for behavioural, histological and SEM investigations.

**Behavioural tests.** It was demonstrated in *A. glomeratus*, that the female ovipositor elicited male's wing vibrating behaviour and other organs failed (Tagawa, 1977). In the present work, a solitary endoparasitoid *A. plutellae* was examined to know what part of the female body was effective in eliciting male's mating behaviour.

Unmated wasps within a week after emergence were used for the tests. At first, each sex was cut into torso and abdomen. Then, the objects were offered to 8 test males. Secondly, after rinsed with ethyl-ether, each sex was cut into torso and abdomen similarly. These objects were then presented to 20 test males. Thirdly, after rinsed with ethyl-ether, the female's ovipositor was pulled out with forceps gently in the water. Then, the terminal portion of the abdomen, including the 9th tergite,

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reproductive system, hind gut and anus was removed. In the previous studies with some *Apanteles* species (Obara and Kitano, 1974; Weseloh, 1976, 1980; Tagawa, 1977), this portion was shown to contain the pheromone source. Various parts of this terminal portion were separated and presented to 11 to 20 test males. Each of the test males was confined in a 30 ml vial.

In preliminary observations, male wasps of all *Apanteles* species investigated showed wing vibrating behaviour against female odour. Thus, the wing vibrating response and copulatory attempts of males were observed at room temperature for 5 min. Light was provided by a cool 20-W fluorescent white tube placed 50 cm above the vial.

**Histological observations.** Adult wasps of seven *Apanteles* species were fixed in Carnoy's fixative for 1 hr. Dehydration was done in routine methods, and the wasps were embedded in Merck's paraffin (m.p. 56–58°C). All sectioning was done at 7 μm thickness. Mayer's haematoxylin and eosin were used for photomicroscopic observations.

**Scanning electronmicroscopic observations.** Among seven *Apanteles* species, *A. kariyai*, *A. liparis* and *A. baoris* were used for this study, because the samples of other species were not large enough to examine.

*A. glomeratus*, a parasitoid of the small white butterfly, *Pieris rapae crucivora*, was also examined. Because SEM observations were not carried out on this species in the previous study (Tagawa, 1977). This wasp was obtained from the parasitized host larvae collected in the field in Japan.

To see the outer surface of the female genitalia, the ovipositor was pulled out with forceps gently in the water, then the terminal portion of the abdomen was removed. Then, it was dried and coated with ca. 40 nm gold in a JEOL ion sputter, and examined in a JEOL JSM-T20 SEM at an accelerating voltage of 12.5 kV. Photographs were taken with Fuji Neopan SS film.

**RESULTS**

**Behavioural tests**

Both the torso and the abdomen of the female were active in eliciting male's wing vibrating behaviour (8/8), and both parts of the male were not effective (0/8). The results were similar to those obtained in *A. glomeratus* (Tagawa, 1977), in which the pheromone activity of the torso was concluded to be due to the contaminated pheromone.

When male or female body parts were presented to males after rinsed with ethyl-ether, most of the males (15/20) responded to the female's abdomen and none to her thorax and to neither part of the male. This apparently suggested the pheromone source existed in the abdomen of the female.

When some parts of the female abdomen were presented to males, more than half of them (13/20) showed wing vibrating response against the 9th tergite and ovipositor, and only one against the ovary and accessory glands mixture. None of the males responded to the other parts. Therefore, the 9th tergite and the ovipositor were separately presented to males, and their response was examined more precisely.

Five of 11 males showed antennation to the ovipositor and the equal number to the 9th tergite, and 4 and 3 of the males exhibited wing vibrating response to the ovipositor and to the 9th tergite respectively. Neither part could elicit copulatory attempts in males.
This implied that the source of the female sex pheromone was on the ovipositor and/or on the 9th tergite.

Histological observations

_A. plutellae_ had a pair of secretory glands on the 2nd valvifer, which was similar to that previously described in _A. glomeratus_ (Tagawa, 1977). The location of the gland is shown in Fig. 1. Every other _Apanteles_ species investigated had similar organs exactly on the same sites as those of _A. plutellae_. Figures 2 to 4 show the organs of some of these species.

The gland was located at the quite near ventral side of the mass of setae (ca. 20) growing at the base of the 2nd valvifer. The organ seemed to be consisted of large secretory cells, each of which had a relatively large nucleus. The cuticle in this region was quite thin and not exceeding 2 μm in thickness. In some cases, tiny porelike structures could be found in this area. The size of the gland was variable with the

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Fig. 1. Photograph (A: ventral view) and schematic illustration (B: side view) of female genitalia of _A. plutellae_.

species examined.

In *A. plutellae*, for example, the length and the width of the gland were about 60 μm and 25 μm respectively. Each cell of the gland was about 10 μm and had a large nucleus (ca. 7–8 μm).

The organ size of *A. lipardis*, *A. baoris*, *A. ruficrus*, *A. kariyai*, A. sp (1) and *A. sp (2)*. was ca. 70 μm × 40 μm, ca. 25 μm × 12 μm, ca. 90 μm × 45 μm, ca. 75 μm × 35 μm, ca. 70 μm × 35 μm and ca. 65 μm × 30 μm, respectively. Among these, *A. baoris* seemed to have smaller glands than others. Cell and nucleus sizes of each species were nearly equal to those of *A. plutellae*. 
The male wasp of every species had, however, no similar secretory organs in the same area.

Present investigations also revealed that a pair of patches of epithelial secretory cells existed on the 9th tergite of A. plutella (Fig. 5), and of other Apanteles species.

In A. melanoscelus, WESLOH (1980) already described that a pair of similar secretory organs existed on the 9th tergite of the female, and that there were no similar ones in the male. Being supported by the results of behavioural tests and histological observations, he concluded that these organs were the pheromone sources in A. melanoscelus. He, however, did not refer whether or not another secretory organs existed on the 2nd valvifers of the female.

In A. plutella, the organs on the 9th tergite could be found in both sexes. And, this was the case in every other Apanteles species examined. Thus, it was not considered the sex pheromone organ.

Scanning electronmicroscopic observations

The surface of the 2nd valvifer of A. kariyai, A. liparis, A. baoris and A. glomeratus are shown in Fig. 6 (A–D). Each of the photograph indicates that tiny pores exist at the ventral side of the mass of setae, corresponding with the locus of the secretory gland.

In A. glomeratus, there were approximately 20 pores, and the sizes of them varied from 0.5 to 1.5 μm (Fig. 6D). In A. kariyai, there were about 15 pores, the sizes of which were from 0.5 to 1.0 μm (Fig. 6A). A. liparis also had about 15 pores, and the size of the pore was nearly the same as the one of A. kariyai (Fig. 6B). A. baoris, however, seemed to have less pores than other three species, and the size of the pore also seemed to be smaller (ca. 0.3 μm) (Fig. 6C).

On the other hand, the pores could not be found on the 9th tergite of A. kariyai and of other species examined.
DISCUSSION

In the ichneumonid wasp *Campoletis sonorensis*, Vinson (1972) reported that all regions of the female body including excised legs and wings had pheromone activities, and that no response in the male was elicited from the various internal organs tested. Thus, he suggested the possibility that the female sex pheromone might be secreted through the cuticle, or by glands associated with the cuticle.

Females of *A. plutellae* had a pheromone activity both in the torso and in the abdomen. Then, the body of the female was washed out with ethyl-ether, because it was already shown that some organic solvents as ethyl-ether, acetone, chloroform etc. could extract sex pheromone of the female in another wasp *A. glomeratus* (Kitano, 1975).
The pheromone of *A. plutellae* is also soluble in ethyl-ether as shown in *A. glomeratus*. After washing out with ethyl-ether, the activity of the torso disappeared. The results suggest that the pheromone activity of the anterior portion of the female is due to the contaminated pheromone. Similar contamination by the pheromone was already reported in *A. glomeratus* (TAGAWA, 1977).

In *C. sonorensis*, too, sex pheromone was soluble in some organic solvents as ethyl-ether, acetone etc., and the female body after being extracted with these solvents lost pheromone activity (VINSON, 1972). When not hardly extracted and lightly rinsed with any solvent, the pheromone activity might remain in the abdominal portion in this species. The results obtained in *C. sonorensis* must be responsible for the contaminated pheromone.

In the braconid wasp *Cardiochiles nigriceps*, VINSON (1978) presented the possibility that a DUFOUR's gland was the source of a sex pheromone, because the males showed antennating behaviour against the DUFOUR's gland and it was most effective. In
A. glomeratus, all of the female's accessory glands failed to elicit male's mating behaviour and the cuticle pieces of the ovipositor were effective (Tagawa, 1977).

In the present study, A. plutellae males (13/20) responded to the female ovipositor and 9th tergite by vibrating their wings, and only one male to the ovaries and accessory glands mixture. The rate of response of the males against the ovipositor was nearly equal to that against the 9th tergite. The results of these behavioural tests on this species suggest that the female sex pheromone source is on the ovipositor and/or on the 9th tergite. This is similar to that obtained in A. glomeratus (Tagawa, 1977), in which the 9th tergite was less effective than the ovipositor. The locus of the pheromone gland must be determined carefully by histological methods.

In A. glomeratus, the pheromone gland was determined to be located at the base of the 2nd valvifer (Tagawa, 1977). Present studies reveal that females of A. plutellae and other 6 Apanteles species examined have similar glands exactly in the same portions of the 2nd valvifers.

Present investigations also manifest that a pair of patches of secretory cells exist on the 9th tergite of A. plutellae female (Fig. 5 A), which is considered to be homologous to that appeared in A. melanosecetus. In A. melanosecetus, a solitary parasitoid of the gypsy moth, Weseloh (1980) reported that the 9th tergite was the source of the female sex pheromone. He found a pair of patched secretory glands on the 9th tergite. He, however, did not refer whether or not secretory glands existed on the 2nd valvifer. In this species, the part of the female abdominal tip which contained the 9th tergite was more effective in eliciting male's mating behaviour than the one containing the 2nd valvifer. By these results, he concluded that the 9th tergite was the pheromone source. The higher pheromone activity of the 9th tergite, however, might be due to the contaminated pheromone, because the female was not washed with any solvent in his study.

Weseloh stated that male A. melanosecetus did not have this type of organs on the 9th tergite. A. plutellae males, however, have similar organs on the 9th tergite (Fig. 5 B). And, this is the case in every Apanteles species examined. Therefore, the pair of glands appeared on the 9th tergite is considered to have another unknown role that is common in both sexes.

If the organs on the 9th tergite were the pheromone glands as Weseloh (1980) suggested, the secretory pores might be found in the cuticle at the site corresponding with the organ. SEM photograph of the female terminal portion of A. kariyai reveals that there are no pores on the surface of the 9th tergite which have epithelial secretory cells underneath.

On the other hand, the 2nd valvifer of this species has some tiny pores at the site corresponding with the secretory gland, just ventral side of the mass of setae (Fig. 6A). SEM photographs of other Apanteles species show the similar figures (Figs. 6B to 6D). This strongly supports that the true pheromone glands of Apanteles group must be the ones located at the base of the 2nd valvifers. The female sex pheromone must be secreted through these pores.

In another braconid wasp Cardiochiles nigriceps, the attempts to find out some secretory organs on the 2nd valvifer was unsuccessful, and the Durfour's gland was effective in releasing male's mating behaviour (Vinson, 1978). The source of the sex pheromone in this wasp might not be the same as the one of Apanteles group.

Behavioural tests to locate pheromone sources in parasitic wasps were difficult
because of much of contamination by the pheromone as occurred in *C. sonorensis* (Venson, 1972), *A. glomeratus* (Obara and Kitano, 1974; Tagawa, 1977) and *A. melanoseclus* (Weseloh, 1976, 1980). Also in *A. plutellae*, there appeared some puzzling results. Histological and SEM observations, however, imply that the response of males to the 9th tergite would be due to the contaminated pheromone.

The pheromone would be secreted through the tiny pores in the cuticle of the 2nd valvifer and contaminate the whole body of the female. And, this would be the case in every *Apanteles* species.

Formerly, in *A. glomeratus*, I thought the pheromone might be secreted from other part, which was the site with the mass of setae, because the gland was large enough to cover this region and the hollows in the cuticle corresponding with the setae were mis-interpreted as pores (Tagawa, 1977). In fact, the main part of the gland was apart from the cells just beneath the setae as Figs. 2 to 4 show.

In *A. glomeratus*, the active pheromone space of a single female is small and about a few centimeters in diameter. Though the male can find and orient to the female by the aid of pheromone (Tagawa and Hitaka, 1982), the pheromone itself is not enough to release the copulatory behaviour in males. It is revealed that a small black object within a few millimeters of the pheromone source is important to release male's copulatory attempt (Kitano, 1975; Tagawa and Hitaka, 1982).

The actual function of the pheromone of *A. glomeratus* in the field was already described (Tagawa and Kitano, 1981).

In general, the males emerge from cocoons a little earlier than the females. The males after emergence are attracted to the cocoon cluster of their own and show wing vibrating behaviour in searching for females, because of the sex pheromone adhering to the cluster.

Active pheromone space of the cluster is about 10 cm in radius, which is larger than that produced by a single female. Females which appear later in this pheromone area have the chances to encounter males which are searching for them. Then, the copulation can be taken place.

In *A. glomeratus*, the sites where successful copulation occurred were almost always within the effective pheromone area, and the ratio of sibmatings was nearly 60% (Tagawa and Kitano, 1981). It was apparent that, in *A. glomeratus*, the contamination of the pheromone to the cocoon cluster had an advantage in increasing the chances of matings.

Contamination of the pheromone might have a similar role in other *Apanteles* species.

The glands at the base of the 2nd valvifers found in *Apanteles* had not been described before Tagawa (1977).

In the abdominal tip of the female honey bee, Koschevnikov (1899) described a pair of glands, the function of which was unknown. According to Hemstedt (1969), the Koschevnikov's gland was located beneath the cuticle of the 8th tergite. And, the gland had some secretory ducts. Such ducts, however, cannot be found in the pheromone gland of *Apanteles*. Because of these differences, the pheromone gland in *Apanteles* cannot be considered the same as the Koschevnikov's gland.
ACKNOWLEDGEMENTS

The author thanks Prof. T. Hidaka of the Kyoto University for his reading the manuscript and his valuable advice. Thanks are also due to Dr. T. Yasuda of the University of Osaka Prefecture for his valuable advice on the morphology of the genitalia. I am also grateful to the members in the same laboratory for their helping the author’s work and offering the parasitized host larvae of some Apaneles species.

REFERENCES


