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Calling Behavior and Sex Pheromone Gland of the Asian Corn Borer, *Ostrinia furnacalis* (Guenee) (Lepidoptera: Pyralidae)

조명나방, *Ostrinia furnacalis* (Guenee) (Lepidoptera: Pyralidae),의 유인행동과 성페로몬샘

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ABSTRACT Adult emergence period, the calling behavior pattern and the mating ability of female depending on ages, 3-day old male's response to sex pheromone released from each 1- to 5-day old female, and the structure and location of sex pheromone gland were observed in *Ostrinia furnacalis*. Adults usually emerged from 1 hour before to 1~2 hours after the beginning of scotophase under the 16 L/8D photoperiod. And most active calling behavior was observed for two hours from the 5th to 7th hours of scotophase among 2- and 3-day old females, and 3-day old male' response to 3-day old females was higher than to any other age of females during the 5th~8th hours of scotophase. Mating frequency was higher at the 4th~7th hours after the beginning of scotophase. Among 1- to 4-day old females extruding sex pheromone gland, more than 65% of them successfully mated with 2- to 4-day old males. And, 2- or 3-day old females extruding sex pheromone gland showed an ability, over 60%, to mate with each 1-~5-day old males. Hypertrophied cell layers, presumed to be a sex pheromone gland of female adults, were located at two intersegmental membranes between the 8th & 9th, and the 9th & 10th abdominal segments.

KEY WORDS *Ostrinia furnacalis* Guenee, calling behavior, sex pheromone gland

초 록 본 연구에서는 조명나방 성충의 우화시간대, 나이에 따른 암컷의 유인행동양상과 교미능력, 1일부터 5일된 암컷이 분비한 성페로몬에 대한 3일된 수컷의 반응 그리고 성페로몬샘의 구조와 위치를 조사하였다. 광주기를 16 L/8D로 했을 때 성충은 일반적으로 암시간대 시작 한시간 이전부터 1~2시간 이후까지 우화 하였다. 그리고 2일과 3일된 암컷에서 암시간대 6시부터 7시 사이에 가장 왕성한 유인행동을 보였으며, 3일된 암컷에 대해 수컷의 반응이 암시간대 5~8 시간에서 가장 높게 나타났다 교미쌍은 암시간대 4~7시간에서 가장 많이 관찰되었다 1일부터 4일된 암컷중 성페로몬샘을 돌출시킨 개체들은 65% 이상의 교미율을 보여 주었다 그리고, 성페로몬샘을 돌출한 2~3일된 암컷은 1~5일된 각각의 수컷과 60% 이상의 교미율을 보여주였다. 암컷의 성페로몬샘으로 추측되는 두꺼운 세포층들은 복부 8번째와 9번째, 그리고 9번째와 10번째 사이의 막질성 표피층에 위치하고 있었다.

검색어 조명나방, 유인행동, 성페로몬샘

The Asian corn borer, *Ostrinia furnacalis*, is a serious pest of corn in China, Taiwan, the Philippines and Japan, and also damages corn, ginger, etc. in Korea. The female-produced sex pheromone was reported to be a blend of the trans- 12-tetradecenyl acetate, cis-12-tetradecenyl acetate in the ratio of 53 : 47 (Kou *et al.* 1992) or 1 : 3 (Yeh *et al.* 1989) in Taiwan, 47 : 53 in China (Cheng *et al.* 1981), 1 : 1 in the Philippines (Klun *et al.* 1980) and 2 : 3 in Japan (Ando *et al.* 1980) based on the analsis by GC-MS and the bioassay in the corn field, but those blends were less attractive than that of virgin

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female in all cases.

What did make such a different ratios of sex pheromone components in the oriental region ? To find out the reason of such a diversity, we compared all experimental methods used in the research of sex pheromone of *O. furnacalis*, which were reported from the oriental region, and found that there are several differences from the extraction step to the bioassay step for sex pheromone. Therefore, we could not treat the diversity of the sex pheromone components' ratio as a geographical variation, though it still has some possibility.

In Lepidoptera, the quantity and/or ratio in sex pheromone components is usually depedent on mating, age, circadian rythm and environmental conditions (Tang *et al.* 1992; Ono 1993; Babilis & Mazomenos 1992), and the calling behavior pattern generally coincides with the pattern of sex pheromone production (Delisle & McNeil 1987; Snir *et al.* 1986). But each *O. furnacalis* used in the experiments of oriental region was under the different environmental conditions. That is, the populations used for sex pheromone extraction were the field-collected or the successive-reared ones in insectary, and rearing temperature was variable from 23 to 27℃ under the 12L/12D ~ constant light condition. In addition, the extractions of sex pheromone were achieved without any information about the calling and mating period in a few cases. All of these different conditions for experimental insects might cause the diverse ratio of sex pheromone componenets in analysis step. In field bioassay, two components' ratio based on the ratio from GC-MS analsis were not so wide range but one or 4 kinds except for test in China, and the results were not so satisfactory to define a precise ratio. Only Cheng *et al.* (1981) treated various ratio from 1 : 92 to 100 : 0 of E12-14 : OAc : Z12-14 : OAc, however, also in this case, the male number caught in the water trap was not significaltly different in most of blends. In Japan, the blend of E/Z12-14 : OAc defined from GC-MS analsis even could not attract any male O. *furnacalis*. And then, all reporter said that other minor components might be needed to attract male successfully and bioassay with wider range of the sex pheromone ratio could suggest a precise sex pheromone ratio.

Therefore, it is indispensible to analyze the Korean population's sex pheromone for the practical application in the Korean corn field. Here we report the calling behavior (extrusion of abdominal tip) pattern of *O. furnacalis* female, male response to sex pheromone released from 1- to 5-day old females, their mating ability depending on ages and the location of the sex pheromone gland to get the available informations for the sex pheromone analsis.

MATERIALS AND MATHODS

Insect

The insects were obtained from a laboratory colony reared under a 16 hours light-8 hours dark regime at 26± 1℃. The insects were sexed on the day of adult eclosion and transferred to the experimental cage (30×30×20 cm) and 10% sugar solution was offered as food. Water was sprayed into the adult-containning cages two or three times a day.

Emergence Times, Calling Pattern and Mating Period

Emergence times of 200 adults were observed from 1hr before the beginning of scotophase to the end of scotophase. To observe the pattern of ovipositor extrusion (calling behavior), 10 female adults were kept individually in a small plastic cup (4ϕ×4 cm) during the scotophase under the dim red light. Observation interval was 1 hour. Replication was seven times for every 1- to 5 day-old females. Females, once extruding the abdominal tip, maintained it in that way until 1 or 2 hours after lighting So newly gland-extruding individuals were checked in every hour. To determine the relationship between calling and mating period, mating pairs from fourty 1 to 2-day old females and fifty males kept in a large cage (150×100×50 cm) were observed with an interval of 30 min. for three consecutive days Mating duration was one or two hours.

Mating Ability of Females at Different Ages

To determine the female mating ability, from two to five (total thirty of each age except for 6- and 7-old female which were fifteen) female individuals extruding ovipositor were applied. according to ages,

into the cages keeping ten to twenty 2- to 4- day old males, and their mating rate during the scotophase was counted with an interval of 30 min. Once mated, the pairs were removed from the experimental cages and another gland-extruding females were applied. And ten to tweenty of males were kept in cages, according to ages, and 2- to 3-day old females extruding ovipositor were introduced in those cages, in a same method described above, to define their mating ability with variably aged-males.

Male Response to Sex Pheromone Released from Females of Different Ages

The system for observation of male response (the extrusion of hairpencil, Yeh *et al.* 1989) to sex pheromone released from variably aged-females is illustrated in Fig. 6. Ten 3-day old males which had been kept in a cage from the emergence to experimental day were transfered to a glass ware before 15 hours of observation, and five virgin females were kept in a flask (500 ml) which was linked to a male-containning glass ware with PVC tube (ϕ1 cm). Air has been flown (35 ml/min.) through activated-charcoal and distilled water, for eliminating contaminants, from setting time to the end of observation. Oservation was conducted under the dim red light, and the condition in a glass ware was 25± 1℃ temp. and 90± 5% RH.

Observation of Sex Pheromone Gland Distribution

Two-day old females were used for observation of sex pheromone gland distribution. Prefixation was carried out by injecting 10 μl fixation solution (5% glutaraldehyde dissolved in 0.05 M phosphate buffer (pH 7.2) containing 2% sucrose and 0 01 M $CaCl_2$ through the 7th abdominal segment (Lalanne-Cassou *et al.* 1977). Then the ovipositor was extruded by pressing the abdomen with a pair of forceps and excised into primary fixation solution (2.5% glutaraldehyde in phosphate buffer). Osmium tetroxide (1%) was used for the post fixation, acetone series for dehydration, embedded in Epon. The abdominal tips embedded were wholly sectioned in 1 μm thickness crossly and longitudinaly, and stained with 0.5% toluidine blue.

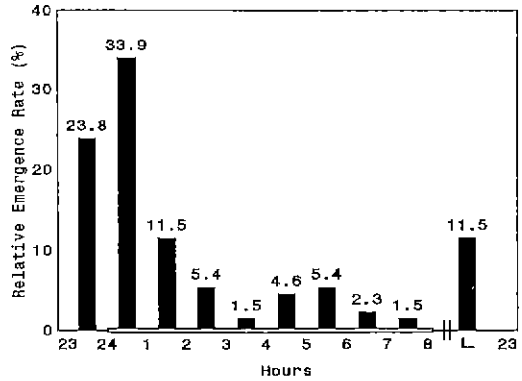

Fig. 1. Emergence pattern of *O. furnacalis* adults in a day (16L/8D). Scotophase is from 24 to 8 O'clock. L: rate of emergence during the light phase from 8 to 23 O'clock

RESULTS

Emergence Pattern

The adults of *O. furnacalis* generally emerged from 1 hour before to 1~2 hours after the beginning of scotophase, accounting for almost 70% (Fig. 1). The rest of them appeared through the entire scotophase and photophase. On exiting from the pupal exuviae, adults moved to a place where they could expand their wings within about 20 min. and adults did not show any active locomotion during the scotophase of the emergence day. From the observation of such a regular pattern of emergence, it is sure that *O. furnacalis* adult has an accurate physiological circadian rhythm which might be applied to the pheromone synthesis and mating behavior.

Calling Pattern during the Scotophase and Mating Period

Calling (extrusion of abdominal tip) pattern of female depending on ages is shown in the Fig. 2. Active calling was observed in 2- to 3-day old females and calling number increased maximally for two hours from 5th to 7th hour of the scotophase. As females got older, the time of the maximum increase in calling number usually advanced by about an

hour a day. But among 5-day old females, such a pattern was not shown, and rather their calling behavior was irregular during the scotophase. When seen as a whole age (from 1 to 4 days), the higher calling activity appeared during three hours, from 4th to 7th hours of the scotophase. We could presume from such a results that the active mating behavior would appear at the same time, because maximal calling activity and pheromone production are usually synchronous in moths (Babilis & Mazomenos 1992). In practical, when we checked the mating pairs from fourty 1- or 2-day old females and fifty 1- or 2-day old males which were housed together in a large experimental cage for three consecutive days, about 65% of mating pairs were observed during 3 hours from 4th to 7th hour of scotophase (Fig. 3), generally coinciding with the calling behavior pattern mentioned above.

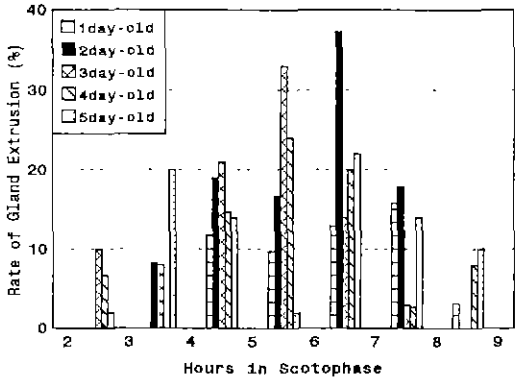

Fig. 2. Rate of initial sex pheromone gland extrusion in *O. furnacalis* female adults during the scotophase under the 16L/8D photoperiod. Number of females observed is 70 at each age

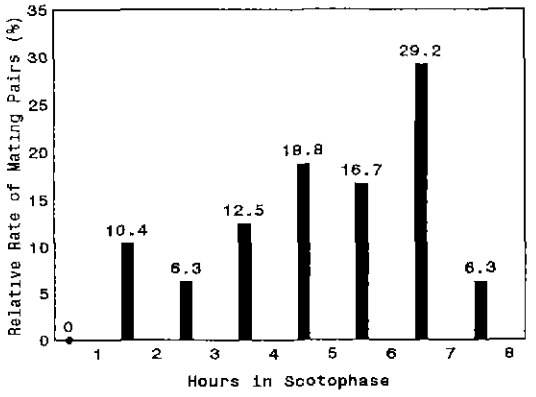

Fig. 3. Number of mating pairs in *O. furnacalis* during the scotophase under the 16L/8D photoperiod (the experiment was lasted for 3 consecutive days, beginning with 1- to 2-day old adults).

Mating Ability of Female Adult

Among 1- to 4-day old females, extruding ovipositor, more than 65% of them successfully mated with 2- to 4-day old males (Fig. 4), but from the 5th day on, mating ability of females decreased considerably. And, mating rate of 2-~3-day old females extruding sex pheromone gland were over 65% with 1- to 5-day old males, but with 6- and 7-day old males, it decreased appreciably (Fig. 5). On the basis

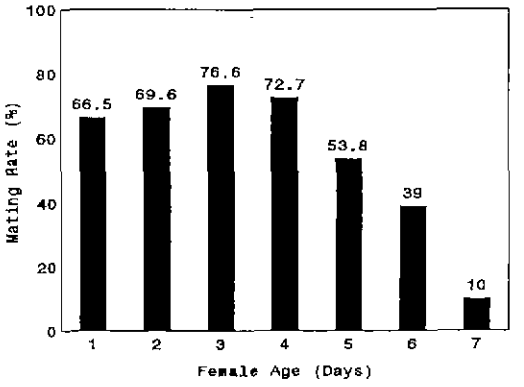

Fig. 4. Mating rate of *O. furnacalis* female adults, extruding sex pheromone gland, with mature male adults (2-~4-day old), depending on ages.

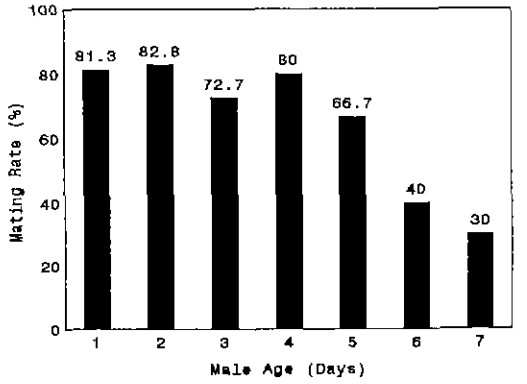

Fig. 5. Mating rate of 2-~3-day old *O. furnacalis* female adults extruding sex pheromone gland with variably aged-males.

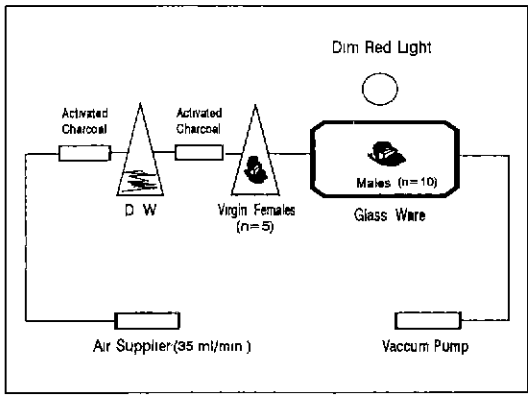

Fig. 6. The system for observation of male response to sex pheromone released from 1- to 5-day old virgin females.

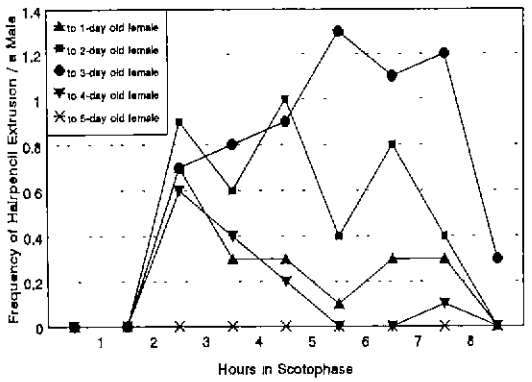

Fig. 7. Response (hairpencil extrusion) of 3-day old male to sex pheromone released from 1- to 5-day old females during the scotophase at 16L/8D photoperiod, 26± 1℃ temp, 90± 5% RH

of this result, we could use 3-day old males, as shown in the middle of high mating ages, for observation of male response.

Male Response to Sex Pheromone Released from Virgin Females

O. furnacalis males usually extrude the hairpencil just before copulation as a response to sex pheromone released from the females, when observed in a glass ware (Fig. 6), and it took 10~30 min for once-responded male to exhibit second response to the continuous stimulation of sex pheromone.

Male response to 3-day old females was stronger than to that of any other aged-females (Fig. 7). That response initiated at between the 2nd and 3rd hour of scotophase, then increased continuously to the highest response period (between 5th and 8th hour of scotophase). To that of 2-day old females, similar degree of male response appeared at the beginning, but maximal response was shown at the 4th hour and then more or less decreased. But, to those of 1- or 4-day old females, male responded once between the 2nd and 3rd hour of scotophase and then the response decreased to a very low level during the scotophase. And 5-day old females could not induce any response of males.

Location of Sex Pheromone Gland

Thick cell layers, presumed to be a sex pheromone gland of *O. furnacalis*, was located at two intersegmental membrane surfaces between 8th & 9th, and 9th & 10th abdominal segments (Fig 8(a)). They are a typical sex pheromone gland of protrusible ring type retracted in the 8th and 9th segments and exposed to the environment only during continuous pulsing of the abdominal segments. The first intersegmental membrane showed only ventrolateral hypertrophied columnar cell layer (G1, about 60 μm thick) (Fig. 8(b)), but the second intersegmental membrane contained a ring gland with ventrolateral hyphertrophied columnar cells (G2, about 60 μm thick) (Fig. 8(c)) and dorsolateral columnar cells (G3, about 40 μm thick) (Fig. 8(d)) which were invaginated more deeply within the body cavity (Fig. 8(a)). And, in G1 and G2 thick endocuticle (about 35 μm thick) overlied and extended into the cell layer, but G3 did not seem to have endocuticle.

DISCUSSION

The pattern of female calling and sex pheromone production in lepidoptera is generally coincided (Delisle & McNeil 1987; Snir *et al.* 1986). The quantity and/or ratio in sex pheromone components is usually dependent on mating, age, circadian rythm and environmental condition (Tang *et al.* 1992, Onc 1993; Babilis & Mazomenos 1992). However, in the virgin females at the same environmental condition, sex pheromone production would be mainly affected by age and circadian rythm. In such a res

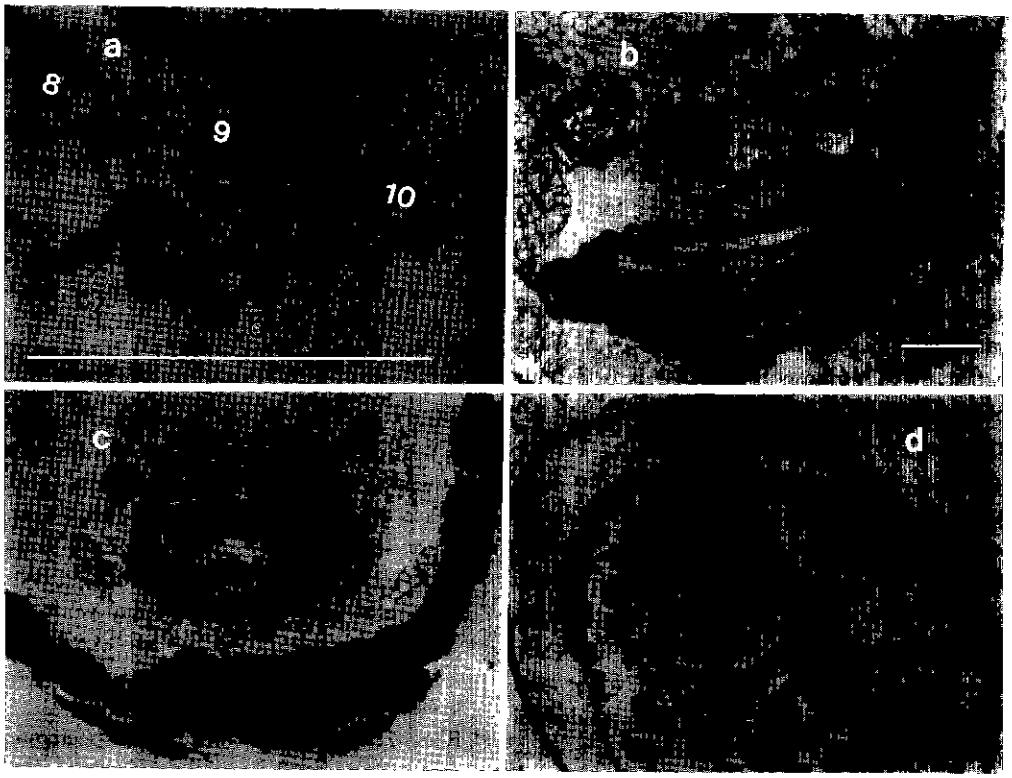

Fig. 8. Sex pheromone glands of *Ostrinia furnacalis* Panel a. longitudinal section of abdominal tip. Panel b: cross section of G1, gland on the ventral surface of the 8th and 9th intersegmental membrane. Panel c: cross section of G2, gland on ventral surface of the 9th and 10th intersegmental membrane. Panel d cross section of G3, gland on the dorsal surface of the 9th and 10th intersegmental membrane, O: oviduct, B: bursa. Scale bar means 1 mm in panel a and 0 1 mm in panel b, c, d

pect, *O. furnacalis* female may produce the higher sex pheromone quantity at 2nd or 3rd day after emergence because calling activity was higher at these days than at any other days, and females would be fully matured at that age because calling is highly correlated with specific stages in development of eggs (Liang & Schal 1993). That is, the total percentage of calling individual among 2- and 3-day old females were almost 90%, but that of 1-, 4-, 5-day old females were 60~70% (Fig. 2). Indeed, we have successfully isolated sex pheromone from virgin females, and found that its amount is higher at 3-day old females (unpublished observation). Therefore, if the higher level of male response to sex pheromone released from 3-day old female is considered together (Fig. 7), 3-day old female would be a profitable one to attract males However, since the mating rates of 1-, 4-, 5-day old females extruding the sex pheromone gland were also high, there might be some other factors to reach the copulation step.

The periodicity of calling was apparant in 2- and 3-day old females during the scotophase, and newly calling number appeared maximally between 6th and 7th, 5th and 6th hour of scotophase, respectively (Fig. 2) And the higher mating frequency for 2- or 3-day old females between 4th and 7th hour of scotophase (Fig. 3) is also related to the calling pattern. Now we are trying to define the relationship between calling pattern and pheromone production at the basis of a circadian rhythm.

High humidity condition is important for reproduction in the *Ostrinia nubilalis* (Loughner & Brindley 1971; Royer & McNeil 1991) which is a geogra-

phical variation of *O. furnacalis*, and the decline in mating success at low relative humidity could be due to reduced adult flight activity (Broersma *et al.* 1976) or female calling behavior (Webster & Carde 1982; Royer & McNeil 1991). It also results from a low receptivity in male (Royer & McNeil 1993). Therefore, to obtain a good condition for calling in female and receptivity in male, we supplied high humidity (90± 5% RH) to the system for the observation of male response by means of constructing the water filtering system.

The first male response between 2 and 3 hour in scotophase could not be explained with the results obtained in this experiments, because the calling activity at that period was low. To explain such a result, further research, such as titration of sex pheromone content during the scotophase, should be conducted. In respect to the structure, hyphertrophied cells at the abdominal intersegmental membranes of this species were a typical lepidopteran sex pheromone gland. And, according to Noirot and *Quennedey's classification* (1974) of epidermal gland, the type of G1, G2, G3 could belong to class 1 which are kown to be in direct contact with the overlaying cuticle which is secreted by themselves. However, the distribution of sex pheromone gland in this species was different from the reports by Cheng *et al.* (1981) and Lee *et al.* (1989) who mentioned that the gland was located only at the 8th & 9th or 9th & 10th intersegmental membrane. Klun (1968) also claimed a ring sex pheromone gland at only between 8th and 9th abdominal intersegmental membrane in *O. nubilalis*. Because female moths, like above examples, generally have one gland, the current picture (Fig. 8) which shows the thick cell layer at two intersegmental membrane of abdomen must be cleared by electron microscopic examination and sex pheromone analsis.

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