ワモンゴキブリにおける性フェロモン生産時期と生産部位

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Site of the Sex Pheromone Production in the American Cockroach, *Periplaneta americana* L.

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Filter papers kept in containers of the female cockroach, *Periplaneta americana* L., are extensively used as a source of sex pheromone extraction. These females began sex pheromone production about 10 days after imaginal ecdysis and the production continued more than 2 months. Although the calling pause of the female *P. americana* was not observed, sex pheromone could possibly be emitted through the anus. Alimentary canal extract was active on sex pheromone bioassay among various parts of the female body. The midgut was found to have higher activity than other parts of alimentary canal.

Midgut epithelium was examined under the light microscope. An apparent difference was observed in midgut epithelium between males and pheromone-producing females. The CHAMPY-fixed midgut epithelium cells of the pheromone-producing females contained small, but characteristically osmiophillic, lipoid granules of unknown nature.

INTRODUCTION

It has been well established that extracts of filter papers kept in containers of female American cockroaches stimulated males to overt sexual response (ROTH and WILLIS, 1952). Extraction of the filter papers has been widely used as the source of the sex pheromone for the isolation regardless of the production mechanism in the virgin females (WHARTON et al., 1954). WHARTON et al. (1957) revealed that newly emerged females produced very little attractant to males, that the attractance reached a maximaml after 2 weeks, and that the production continued for several months afterwards.

However, STÜRCKOW and BODENSTEIN (1966) have reported that the severed head of female American cockroaches elicited sexual response from males, and thus they postulated the site of production to be located in the head. Later, one of above authors reported that sex pheromone was produced in the alimentary tract and maximal activity was obtained from crop and midgut extracts when females were 9 to 11 days after imaginal ecdysis and the crop contained a quantity of food (BODENSTEIN, 1970).

Study of the site of pheromone production relevant to initiation of production has attracted as much interest as chemical study of the sex pheromone. Our attempt to observe calling pose and successive mating of the attractive (sex pheromone producing) females has so far been in vain. For these reasons, this experiment has been undertaken to answer the questions when and where the sex pheromone is produced.

MATERIALS AND METHODS

Experimental insects. The American cockroach, *Periplaneta americana* L., was reared at 25–28°C and 60% R. H. with mouse food and water. Females were segregated from larvae and males after imaginal ecdysis. Newly moulted adults were designated as 1-day-old females.

Bioassay of the sex pheromone extracts. The bioassay method used for evaluation of sex pheromone activity followed that outlined in a previous paper (TAKAHASHI and KITAMURA, 1972). For comparison of the activity of the extracts, 1 ml ethereal solutions including one female equivalent of alimentary tract portions and body surface washing were prepared. For faeces and filter papers from female jars, 1 ml ethereal solution equivalent to 1 female per day was prepared.

Ten to one hundred microliter of the above solution on a glass plate (2×2 cm) were used in each bioassay.

Extraction of female alimentary tract. The alimentary tract was removed from a female body by dissection and cut into 3 portions—crop, midgut and hindgut. Each portion was extracted with methylene chloride.

Extraction of faeces and filter paper. Each 1-day-old female was kept in a glass jar (12 cm diameter, 7 cm height) lined with filter paper (10×20 cm) and the floor made of wire mesh with segments of 3×3 mm. The jars and the filter papers were changed every day and the faeces underneath the wire mesh were collected. The faeces and filter papers were extracted with methylene chloride. The same methods were applied to those females reared only with water.

Surface washing and extract of females. Females were washed thoroughly with ether, and washed again after removing the wing and tegmina. The washed bodies were cut into head, thorax and abdomen. Each portion was extracted with methylene chloride overnight.

Ligature of females. One-day-old females were kept with food and water for 4 days. Each female was ligatured between thorax and abdomen with string. They were kept in a glass jar with wire mesh floor for more than a month.

Ejaculated faeces of females. Each female was seized with forceps and the soft faeces ejaculated immediately after seizure were collected on a glass-rod (5 mm in diameter, 15-cm long). The glass-rod was bioassayed directly.

Observation of midgut tissue with a light microscope. Midguts were removed by opening the abdomen, pulling the alimentary tract out and clipping off the middle part. The detached midgut was transfered to BOUIN's fixative or CHAMPY's fixative, and then embedded in paraffin. The sections were stained with either MALLORY's triple stain or haematoxylin-eosin.

RESULTS

Bioassay results of extracts of filter papers and faeces from fed, starved and ligatured females of different ages are shown in Table 1. Filter papers placed in glass jars with 1 female each fed or starved showed strong activity when they were 10 days after imaginal ecdysis, whereas the filter papers from ligatured females did not show activity for as long as 30 days. The faeces collected from the jars were found to be active 5 days longer than the filter papers, but had almost the same extent of activity. However,

Table 1. Male Sexual Response to Extracts of Filter Papers and Faeces from Females of Different Ages and Conditions

Extracts		Days after imaginal ecdysis							
		~9	10	11	12	13	14	15	16~30
1♀	faeces	−	−	−	−	−	−	+	++
	filter paper	−	++	++	++	++	++	++	++
2♀	filter paper	−	++	++	++	++	++	++	++
Starved ♀	filter paper	−	++	++	++	++	++	++	++
Ligatured ♀	faeces	−	−	−	−	−	−	−	−
	filter paper	−	−	−	−	−	−	−	−
♀	ejaculated faeces	−	−	−	−	++	++	++	++
♂♀	filter paper	−	−	−	−	−	−	−	−
♀/♂♂	filter paper	−	++	++	++	++	++	++	++

Table 2. Male Sexual Response to Extracts of Various Parts of the Female Body of Different Ages

Extracts	Days after imaginal ecdysis						
	~9	10	16	18	25	30	30~
Head	−	−	−	−	−	−	−
Thorax	−	−	−	−	−	−	−
Abdomen	−	+	+	+	+	+	+
Surface washing	−	−	−	−	−	+	+
Alimentary canal	−	++	++	++	++	++	++
Genital organ	−	−	−	−	−	−	−
Cuticula and fat body	−	−	−	−	−	−	−

faeces and filter papers were inactive through 1 month or more, when each female and male were reared together. When the matured males were kept around a matured female in a small wire cage (designated as ♀/♂♂ in Table 1), the female produced as much pheromone as an isolated female. Matured males isolated from females for more than 1 month successfully mated with 11-day-old females and 30-day-old females, respectively, but not with females younger than 5-day-old, when each pair was confined in a box (23×29×11 cm). This observation coincided with the appearance of activity in the filter papers from female cages.

Bioassay results of the various female parts are shown in Table 2. Surface washing showed weak activity after 30 days, whereas the abdominal extracts were active after 10 days. The abdomen was dissected to alimentary tract, genital organs and the remainder (mainly cuticle and fat body). Since only the alimentary canal was active, this was further dissected to crop, midgut and hindgut. Midgut showed activity, whereas both crop and hindgut were inactive. Activity of midgut was also confirmed by the results that the 11-day-old female had higher activity in the midgut than colon and rectum (Table 3). When the fed and starved females were seized with forceps, soft faecal matters were extruded immediately. The faeces had a strong characteristic smell and were active after 13 days. When two females were reared together, filter papers in the jar were active on the 10th day as same as in the single female. All ex-

Table 3. SEX PHEROMONE ACTIVITY OF FEMALE ALIMENTARY CANALS IN DIFFERENT AGES

Age of females (Days after imaginal ecdysis)	Extracts			
	Foregut	Midgut	Colon	Rectum
1	−	−	−	−
3	−	−	−	−
5	−	−	−	−
11	−	++	+	+
30	−	++	−	−
36	−	++	−	−
39	−	++	−	−

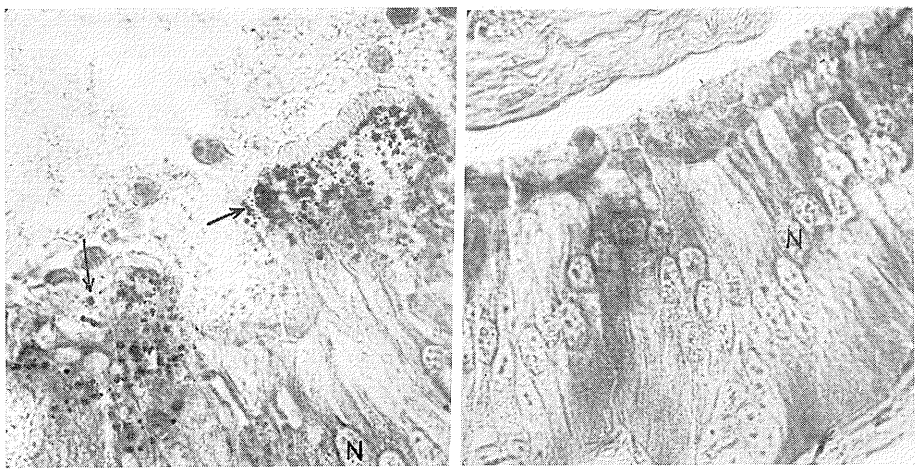

Fig. 1. Midgut epithelium fixed with CHAMPY's fixative. Left: female; Right: male. Arrow indicates dark granules. N: nucleus.

Table 4. OBSERVATION OF THE OSMIOPHILIC DARK GRANULES IN MIDGUTS OF DIFFERENT CONDITIONS

Group[a]	Sex	Dark granules[b]				Total
		++	+	±	−	
1	♀	1	0	0	1	2
2	♀	1	0	0	1	2
	♂	0	0	0	2	2
3	♀	1	4	1	0	6
4	♂	0	0	0	6	6

[a] Group 1, 15-day-old females; group 2, 15-day-old males or females; group 3, more than 30-day-old females; group 4, more than 30-day-old males.

[b] ++, numerous in number; +, presence observable; ±, very seldom observable; −, unobservable.

periments were carried out at 25°C and temperature below 25°C delayed the sex pheromone production.

Examination of midgut sections of males, active females and inactive females with a light microscope revealed no detectable difference among them, when they were fixed in BOUIN's fixative and stained with either haematoxylin-eosin or MALLORY's triple stain. The epithelium was composed of very elongated columnar cells with microvilli at the luminal surface. The CHAMPY-fixed midgut epithelial cells of the females more than 30-day-old, in active stage of pheromone production, however, contained small, but characteristically osmiophilic, black granules in their cytoplasm (Fig. 1), though the number of granules varied considerably from one individual to another. A few 15-day-old females, in early stage of pheromone production, exhibited presence of the granules, whereas the males did not show any sign of presence of such granules in their midgut tissues (Table 4).

DISCUSSION

Pheromones are usually produced by exocrine glands, but this concept does not apply in all cases. A few insects are known in which pheromones arise from a glandualr source associated with internal organs whose prime function is not pheromone production. The hindgut of the boll weevil and various bark beetles has been implicated as the site of production but no conclusive evidence has as yet been reported (PITMAN et al., 1965; SCHNEIDER and RUDINSKY, 1969; HARDEE, 1970; RUDINSKY, 1970). In *Dacus tryoni* (FROGG.), sexually mature males release sex pheromone through the anus (FLETCHER, 1969). This was stored in a secretory sac and reservoir, associated with the posterior region of the rectum. The site of production of the aggregation pheromone of the German cockroach, *Blattella germanica* L., was considered to be a part of alimentary canal, the rectum pad (ISHII and KUWAHARA, 1967).

Search for the sex pheromone production site of the American cockroach has been very controversial because there is neither direct evidence of secretory gland cells nor observation of calling pose, as has been shown to exist in many lepidopterous insects. From our bioassay results of extracts of various sources in different female ages, we found the cuticle was not a secretory part. Secondary contamination of body surface may result in active surface washings after a prolonged rearing period, mostly in groups. Higher activity of female midgut after 10 days in our results coincided with the results of BODENSTEIN (1970), except on the effect of starvation. Moreover, females ligatured between thorax and abdomen did not emit pheromone up to 30 days, but became active after deligature. By tightening of the anterior part of the crop, emission of the pheromone was shown to be prevented, and eventually this provided proof that the pheromone could not be emitted from thorax and/or head, as described by STÜRCKOW and BODENSTEIN (1966). However, the effect of the corpora allata on the pheromone production was not investigated, although this has been suggested by several workers (BARTH, 1961; ROTH and BARTH, 1964). After about 15 days, only the midgut extract showed activity, but on 11th day, just at starting of production, the colon and rectum had slight activity. This fact suggests that pheromone may remain partly in the hindgut at the highest production, but pheromone emittance would not be simultaneous with defecation. This was further supported by finding that inactive faeces were obtained even in the pheromone-producing period.

BODENSTEIN (1970) has suggested that the pheromone productive site may exist in the midgut, but he did not identify the difference in the tissue of active (pheromone-

producing) and nonactive females. Our results using BOUIN-fixation agreed with his result, but not with the result of CHAMPY-fixation. Presence of the granules was found in 6 active females out of 6 dissected, but not in 6 males out of 6 dissected. As to the results of histological observations, we have no positive evidence showing either that the osmiophilic granules found in the midgut cells of the active females may represent the pheromone-carriers or that pheromone would be discharged into the lumen through the luminal surface of the cell. Nevertheless, it is worthy to note that the granules were observed only in the active females, though their numbers varied considerably from one individual to another (Table 4). Strong osmiophilic property of such granules may suggest that they would be lipoidal in nature.

The presence of an electron-dense body in midgut epithelium has been already reported as autophagic vacuoles by COUCH and MILLS (1968) in the starved female adult of *P. americana*. Unfortunately, however, only the females of unspecified age were used in their observation, thus it seems to be difficult to direct correlation of our observations to theirs in detail. We found starvation gave no effect on the pheromone production. As pointed out by NOIROT and QUENNEDY (1974), the presence of characteristic lipoid granules within the cytoplasm of pheromone-producing cells has been reported in several insects. Consideration of all such data, including our present data of the characteristic appearance of osmiophilic granules in the active females of *P. americana*, may suggest at least the presence of enhanced or specialized lipid metabolism in the midgut cells of such animals. As most pheromones are lipoidal in nature, our observations are noteworthy though further studies are required to elucidate the production mechanism.

Filter papers obtained from a colony of females, males and nymphs in a cage did not elicit response on males. Under this condition, we consider that sex pheromone may not always play a primary role in successful mating of the American cockroach.

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