Secretion of Dispersion-Inducing Substance by the German
Cockroach, *Blattella germanica* L.
(Orthoptera : Blattellidae)

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Preference tests were carried out to examine the influence of contamination with different number of adult German cockroach, *Blattella germanica*, on the behavior of nymphs of the same species. The results revealed that the adult cockroach secreted not only aggregation pheromone, but also dispersion-inducing substance.

Aggregation response of the nymphs was found to the filter paper that had been contaminated by 5 or 10 adult cockroaches in a 200-ml test beaker. Intensity of the aggregation increased according to the length of time for contamination.

Whereas, repellency response of the nymphs was found with the filter paper thoroughly wetted by the larger number of 50 or 100 adult cockroaches in a beaker, on the inner wall of which clear droplets had been deposited by the cockroaches. Intensity of the repellency seemed to be increased with the degree of wetness of the paper, but reduced obviously when the discharges were removed from the mouth parts of the cockroaches. The clear droplets, or salivary secretions, were secreted from the mouth parts of the adult American cockroach, *Periplaneta americana*, as well as the German cockroach when they were seized or disturbed. The saliva of both cockroaches showed definite repellency against the nymphs of *B. germanica*.

These results led us to the conclusion that the cockroaches secrete repellent(s) in the saliva, which would act as the chemical stimuli for dispersion from highly crowded area or for warning against their predators.

INTRODUCTION

Pheromones excreted with feces provide a stimulus for aggregation in several species of cockroaches (ISHII and KUWAHARA, 1967; ISHII, 1970; BELL et al., 1972; ROTH and COHEN, 1973; METZGER and TRIER, 1975). However, these pheromones are not only strictly species-specific, but in a few instances certain species do not aggregate to their own feces, or some are repelled by fouling of the other species (ISHII, 1970; BELL et al., 1972; ROTH and COHEN, 1973). ROTH and COHEN (1973) also suggested that the behavior of the nymphs could be influenced by differences in the degree of contamination of test paper. But no experimental evidence has been available on such an assumption so far. In the present experiment, we examined if the population density affects the pheromone secretion by *Blattella germanica* (L.), and/or the contamination with high density generates any repellent factor against the nymphs.

MATERIALS AND METHODS

The German cockroach, *Blattella germanica* L., was used in most of the experiments, but the American cockroach, *Periplaneta americana* L., was also used in some experiments. These two species were reared in our laboratory by feeding dried mouse food (Nippon Clea Co. Ltd.) in plastic cages (20×38×28 cm) as described previously (SUTO et al., 1978). The adult cockroaches were used for contamination experiments and as source of oral secretions. The first-instar nymphs of *B. germanica* were used for the bioassay.

A disk of filter paper (Toyo Roshi Co. Ltd., No. 5c, 5.5 cm in diameter) was contaminated with 5 to 100 adult German cockroaches for 0.5 to 18 hr at 25°C in a glass beaker, 6 cm in diameter, 8.5 cm in height and 200 ml in capacity. After being contaminated, the filter paper was cut into two parts and subjected to the bioassay. Oral secretions were collected into capillaries by picking up the adult individually with forceps, diluted serially with distilled water; and one-tenth ml of the diluents was used to impregnate a small piece of filter paper (Toyo Roshi Co. Ltd., No. 51, 2×5 cm). In some experiments, oral secretions were removed by absorbing with pieces of filter paper.

The bioassay method of ISHII and KUWAHARA (1967) was employed to test the presence of aggregation pheromone or repellent(s). Two pieces of filter paper folded in W shape were placed vertically in opposite positions on the bottom of a glass pot of 9.5 cm in diameter and 5.5 cm in height. One of the papers in each pot had been contaminated under certain condition or impregnated with a sample to be tested. Another clean paper of the same size was used as control. Ten nymphs were released in the center of a pot, and kept overnight. Next morning the numbers of nymphs on the test and control papers were counted. The test usually started at 17:00 to 19:00.

Aggregation indexes were calculated according to ROTH and COHEN (1973), as follows:

$$\text{Aggregation index} = \frac{A-B}{A+B}$$

where A and B are the numbers of nymphs found on treated and control papers, respectively. Index values range from +1 to -1. Positive values indicate aggregation on treated papers, and negative ones show repellency. Values around zero indicate inexact tendency to aggregate or repel.

RESULTS

Secretion of aggregation pheromone and repellent(s) by adult German cockroaches

Experiment 1: Groups of 5 to 100 adult cockroaches were put into test beakers for 18 hr at 25°C. The results are given in Table 1. The blank test with two uncontaminated papers showed no definite tendency to aggregate or repel; the nymphs gathered randomly on the two papers. When the nymphs were given a choice between the two papers, one contaminated with 5 or 10 adult cockroaches and the other as control, they apparently preferred the contaminated paper. However, they did not show preference to the paper contaminated with 20 cockroaches. Toward the papers contaminated with 50 or 100 cockroaches, no aggregation but repellency re-

sponse was observed.

These unexpected results suggest that the adult cockroaches secrete the aggregation pheromone when they are in low densities, whereas in the higher densities they may not secrete the pheromone. It is also possible that they may secrete certain repellent(s), or both the pheromone and repellent(s). These possibilities were examined in the following experiments.

Experiment 2: After contamination with groups of 10 or 100 adult cockroaches for 18 hr, feces of the cockroaches in the beaker were collected and affixed with carboxymethyl cellulose to clean filter papers. The mean amounts of feces pasted were 1.92 ± 0.46 mg (mean $\pm$ s.e.; range, 1.45–2.72 mg) and 4.12 ± 2.38 mg (range, 1.00–8.00 mg) for 10 and 100 adult cockroaches, respectively. Preference tests were carried out also with filter papers placed on the bottom of the beakers.

Table 1. EFFECT OF CONTAMINATION BY DIFFERENT DENSITY OF ADULT *B. germanica* ON AGGREGATION RESPONSE OF NYMPHAL *B. germanica*

No. of adults for contamination	No. of tests	Number of nymphs aggregated on filter paper		χ^2 test ^a	Aggregation index ^b (mean $\pm$ s.e.)
		contaminated	control		
0 ^c	12	67 ^d	53	$p > 0.05$	0.117 ± 0.260
5	12	101	15	$p < 0.001$	$+0.773 \pm 0.166$
10	12	93	18	$p < 0.001$	$+0.691 \pm 0.102$
20	18	82	85	$p > 0.05$	$+0.019 \pm 0.193$
50	19	61	104	$p < 0.002$	-0.256 ± 0.181
100	20	27	166	$p < 0.001$	-0.702 ± 0.083

^a Difference was tested between the numbers of nymphs aggregated on contaminated and control papers.

^b Positive values indicate aggregation on contaminated paper, negative ones show repellency.

^c Test was performed with two uncontaminated papers, one of which was marked with a pencil.

^d Number of nymphs aggregated on filter paper marked with a pencil.

Table 2. PREFERENCE TEST BETWEEN FILTER PAPERS CONTAMINATED OR PASTED WITH FECES OF ADULT *B. germanica* AND CONTROL FOR THE FIRST-INSTAR NYMPHS OF *B. germanica*

No. of adults for contamination	Samples tested	No. of tests	Number of nymphs aggregated on filter paper ^a		Aggregation index ^b (mean $\pm$ s.e.)
			treated	control	
10	Feces	8	77	8	$+0.800 \pm 0.200$
	(1.92 ± 0.46 mg) ^c Paper ^d	12	110	11	$+0.817 \pm 0.122$
100	Feces	10	89	18	$+0.656 \pm 0.145$
	(4.12 ± 2.38 mg) ^c Paper ^d	10	15	87	-0.715 ± 0.092

^a All differences were significant beyond $p < 0.001$ level.

^b Positive values indicate aggregation on treated paper, negative ones show repellency.

^c The mean amount of feces pasted to filter paper (mean $\pm$ s.e.).

^d Filter paper placed on the bottom of the contaminating beaker.

As shown in Table 2, the nymphs preferred the filter papers pasted with the feces or those experimentally contaminated with 10 adult cockroaches. But they were repelled from the filter papers contaminated with 100 adult cockroaches.

These results apparently indicate that the adult German cockroaches secrete aggregation pheromone in the feces irrespective of population densities examined. It is also suggested that they secrete repellent(s) under the higher population densities. Therefore, the origin of repellent(s) was further studied by changing the contamination methods.

Experiment 3: As shown in Fig. 1, the filter papers contaminated with 10 adult cockroaches for more than 3 hr were effective for eliciting the aggregation response. Intensity of the aggregation increased with the length of time of contamination. On the other hand, the filter papers contaminated with 100 adult cockroaches for 0.5 hr elicited apparent repellency response. Intensity of the repellency rather decreased slightly with increase of the contamination periods.

During the contamination with 100 adult cockroaches, it was noticed that clear droplets were deposited on the inside wall of the test beakers, and that the filter papers placed on the bottom became wet at the periphery. But the papers contaminated with 10 adult cockroaches did not noticeably get wet. So, these droplets were considered to contain some repellent(s).

Experiment 4: Groups of 100 adult cockroaches were used repeatedly to contaminate series of filter papers in successive orders as shown in Table 3. The filter papers placed on the bottom of test beakers became thoroughly wet during the first (0.5 hr) and second (2 hr) contaminations, but slightly during the fourth (18 hr). The nymphs apparently repelled the papers contaminated for the first 0.5 hr and

Fig. 1. Time course of secretion of the aggregation pheromone and repellent(s), when contaminated with 10 (■) and 100 (●) cockroaches. Positive values indicate aggregation on contaminated papers, and negative ones show repellency. Vertical bars represent standard errors of the means. Numerals in parentheses indicate number of tests.

second 2 hr. But the intensity of repellency decreased gradually in the third and fourth contaminations. Thus, the intensity of repellency seemed to have a relation with the amount of the clear droplets deposited by the cockroaches.

Experiment 5: The adult German cockroach discharges clear droplets from the mouth parts when seized at the body. To examine the effect of the discharge as regards the repellency observed above, they were starved for 2 days in a rearing cage and picked up individually with forceps. Oozing droplets from the mouth parts were removed by absorbing with pieces of filter paper. Then the cockroaches in a group of 100 were used to contaminate filter paper successively in the same way as in the previous experiments. The results are given in Table 4.

Filter papers placed on the bottom of test beakers and contaminated by the drop-let-deprived cockroaches did not become thoroughly wet. The intensity of repellency of these papers were much reduced in comparison with the results shown in Table 3. These results suggest that the droplets discharged from the mouth parts of the adult cockroaches contain repellent(s) to the nymphs.

Table 3. EFFECT OF SUCCESSIVE CONTAMINATION OF FILTER PAPERS WITH 100 ADULT *B. germanica* ON SECRETION OF REPELLENT(S)

Contami- nation number	Periods of contamination (hr)	No. of tests	Number of nymphs aggregated on filter paper ^a		Aggregation index ^b (mean±s.e.)
			contaminated	control	
1	0.5	12	8	128	-0.886±0.053
2	2.0	12	16	118	-0.752±0.116
3	2.0	10	27	86	-0.575±0.170
4	18.0	12	35	98	-0.326±0.241

^a All differences were significant beyond $p<0.001$ level.

^b Negative values indicate repellency against contaminated paper.

Table 4. EFFECT OF REMOVAL OF DISCHARGES FROM THE MOUTH PARTS OF ADULT *B. germanica* ON REPELLENCY

Contami- nation number	Periods of contamination (hr)	No. of tests	Number of nymphs aggregated on filter paper ^a		Aggregation index ^b (mean±s.e.)
			contaminated	control	
1	0.5	12	55	66	-0.109±0.068
2 ^c	2.0	12	38	88	-0.404±0.056
3	2.0	10	46	55	-0.064±0.086
4	18.0	10	39	52	-0.123±0.062

Preference test was conducted between contaminated and clear filter papers. The former was contaminated with 100 adult cockroaches which had been starved for 2 days and deprived of discharges from the mouth parts, and the latter for control. The first-instar nymphs of *B. germanica* were used as test insects.

^a Differences were not significant (except c) at $p=0.05$.

^b Negative values indicate repellency against contaminated paper.

^c Significant at $p<0.02$.

Fig. 2. Repellency of saliva of *B. germanica* and *P. americana* against nymphs of *B. germanica*. A piece of filter paper (2×5 cm) was impregnated with one-tenth ml of diluted saliva of *B. germanica* (●) or *P. americana* (■), air-dried, and then assayed. Negative values indicate repellency of the impregnated paper. Vertical bars represent standard errors of the means. Numerals in parentheses indicate number of tests.

Repellency of saliva of *B. germanica* and *P. americana*

The adult American cockroaches also secreted clear droplets when they were seized with forceps at the abdomen. The mean amounts of clear droplets secreted by a female were $2.1 \mu\text{l}$ and $15.2 \mu\text{l}$ for *B. germanica* and *P. americana*, respectively. But the amount varied by physiological states of the cockroaches, such as sex, age, crowding condition, starvation, etc. In preliminary experiments, the following materials showed repellency against the nymphs; clear and turbid oral secretions, crop content, and saliva from the salivary reservoirs. The clear secretions appeared to be identical with saliva, since clear droplets were found only in the salivary reservoirs upon dissection of the cockroaches, and turbid secretions vomited by a few of these cockroaches seemed to be counterflows of the crop contents.

The results of quantitative assay demonstrated that the minimal amounts that could elicit definite repellency response of the nymphs were $0.63 \mu\text{l}$ and $10 \mu\text{l}$ per filter paper for the saliva of *B. germanica* and *P. americana*, respectively (Fig. 2).

DISCUSSION

Cockroaches have a variety of glands which secrete several biologically active substances, some of which are concerned in sexual or aggregation behavior. Other secretions produced by both sexes of the adults and nymphs are emitted or ejected when they are disturbed. On the function of the latter secretions, defensive roles have been postulated (ROTH and WILLIS, 1960), and later proved in some cases (WALLBANK and WATERHOUSE, 1970; TAKAHASHI and KITAMURA, 1972; ROTH and ALSOP, 1978).

In the present study, the aggregation response of the nymphal German cockroaches was confirmed with the filter papers contaminated by 5 or 10 adult cockroaches (Tables 1, 2; Fig. 1). The intensity of the response increased with the length of contamination time (Fig. 1). These results are in good agreement with ISHII's observa-

tion (ISHII, 1970).

However, the present study added a new but conflicting finding that the higher density of the contaminating adult cockroaches induced repellency response of the nymphs. The repellency response was found with the filter paper wetted thoroughly by contaminating with 100 adult cockroaches in a beaker, on the inner wall of which were many deposits of clear droplets. Thus, when the cockroaches are confined in an overcrowded arena, they overlap and trample each other, and secrete these droplets that seem to contain repellent(s). Although the aggregation pheromone might be also secreted in such a condition, its function seemed to be less apparent than that of the repellent(s). The degree of wetness of the filter paper seemed to have some relation with the intensity of repellency response; the intensity was reduced by removing discharges from the mouth parts (Tables 3, 4). Moreover, saliva of both *B. germanica* and *P. americana* adults showed definite repellency against *B. germanica* nymphs (Fig. 2). These results led us to the conclusion that these cockroaches secreted repellent(s) in their saliva when they were disturbed.

The saliva of cockroach is generally clear, watery, and approximately neutral, and used for moistening their food in the first step of feeding. Digestive enzymes, such as amylase and invertase, and mucous component are secreted from the salivary glands (WIGGLESWORTH, 1972). In addition, it has been suggested by PAVLOVSKII and SHTEIN (1931) that cockroach saliva contains some biologically active constituents. They studied the effect of experimentally induced bites of *Blatta orientalis* on human skin and noted a degradation of epithelial cells, necrosis, and inflammation. They attributed these changes to a toxic effect of the saliva, since they found that the saliva, when rubbed into the skin with a needle, produced symptoms similar to the bites of the cockroaches. We also found similar toxic effects on human skin with the sterilized saliva of *B. germanica* and *P. americana* (unpublished data). These toxic effects of the saliva may play a defensive role against predators in nature.

WHARTON et al. (1968), on the other hand, noticed salivation of *P. americana* nymphs during their ritualistic encounter behavior, which involved display and physical contact. The saliva secreted in the initial phase of their contact could be interpreted as a chemical signal for the mutual recognition.

Recently, deposition of an aggression-stimulating pheromone has been demonstrated on cotton wicks inserted in vials serving as water source for adult *P. americana* (RAISBECK, 1976). This pheromone affects some individuals of mixed ages, and causes universal aggressive responses followed by dispersion over the floor of test arena. The pheromone seemed to be secreted from an exocrine gland in the head when the cockroaches were prevented from coming to contact with their feces.

Though no aggressive behavior was noticed in our experiments, repellency behavior of *B. germanica* nymphs to the saliva was similar to that of *P. americana* to the aggression-stimulating pheromone: When a group of the nymphs was released in a bioassay pot, they wandered for a while and then some of them approached the contaminated paper, moved about on the paper touching it with their palpi and antennae, then they left the paper and gathered on the control paper. Thus, repellent(s) found in the saliva will serve as a chemical stimulant for dispersion of the nymphs from the highly crowded area. It is possible that the repellent(s) functions as a warning substance among the cockroaches or as a defensive alarm against predators, since both sexes of the adults and nymphs salivate when they are overcrowded, picked up,

anaesthetized, or crushed.

According to ROTH and ALSOP (1978), defensive glandular systems in the cockroaches, so far reported, were classified into five types principally on the bases of their location in the cockroach: 1) paired eversible pleural glands, 2) single ventral intersternal gland, 3) paired dorsal intertergal glands, 4) paired tracheal glands, and 5) hypodermal tergal glands on the terminal abdominal segments. The results of the present experiments have revealed that the salivary glands belong to the sixth type.

Studies on the chemical nature of the repellent(s) are in progress.

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