Sex Discrimination by Males of the German Cockroach, *Blattella germanica* (L.) (Orthoptera : Blattidae)

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Males of the German cockroach, *Blattella germanica*(L.), raise their wings when they touch sexually matured females with their antennae. They then secrete a seductive fluid from their tergal gland upon the females. Antennae isolated from the females still possessed such potency as the wing raising of the males.

Results of structural observation and biological assays with the antennae indicated that factor(s) responsible for the wing raising is principally not physical, but is of a chemical nature, and this factor is localized not only in the antennae of the sexually matured females but also in other organs such as the legs and wings.

This factor seemed to be a kind of pheromone brought about by contact chemoreception of the antennae of the male.

INTRODUCTION

It has been known that males of the German cockroach, *Blattella germanica* (L.), raise their wings when they come in contact with females, and then they secrete a pheromone from their tergal gland. While the female licks the pheromone, the male orients to copulate.

Wing raising of the male is one of the indispensable steps in courtship, since the female is unable to copulate without licking the pheromone secreted from the tergal gland of the male.

ROTH and WILLIS (1952) have studied extensively the courtship behavior of cockroaches, and found that the male of the German cockroach is sexually stimulated by antennal contact with the isolated female antenna of the same species. They concluded that the stimuli involved in the sexual behavior of the males of the German cockroach are both mechanical and chemical. And also they postulated that sex discrimination by males is mainly due to contact chemoreception.

The present experiments were undertaken to know the sequence of courtship of the German cockroach and to confirm whether or not sex discrimination by males is due to contact chemoreception through their antennae.

OBSERVATIONS AND EXPERIMENTS

Observation of courtship sequence

When a sexually matured male meets a female, they contact each other with their antennae and vibrate the antennae. If the male can distinguish the female, he wags his abdomen right and left several times and turns the direction of his ab-

domen to face the head of the female, and then raises his wings. The pheromone is secreted from the tergal gland located in the 7th and 8th abdominal tergites of the dosal side. The female licks this secreted pheromone. During licking of the pheromone, the male steps back and grasps the genitalis of the female by his clasper, and then the male copulates with the female. This secretion is considered to be a kind of sex pheromone secreted by the male. ROTH and DATEO (1966) proposed the name "Seducin" for this pheromone consequent to their study on *Nauphoeta cinerea* OLIVIER.

Role of visual sense in courtship

Five pairs of virgin females and males were reared in a plastic box, 26 cm × 20 cm × 10.5 cm, under darkness for 13 days at 25°C in order to know whether or not copulation takes place without light. It has been observed that more than 90% of the newly emerged females copulated within 10 days after emergence (UEDA *et al.*, 1969). After 13 days, the males were removed, while the females were continued to be reared at 25°C with 14 hr of illumination per day until the eggs hatched. Date of hatching and the number of larvae hatched from oothecae were recorded. Results were as follow:

Number of viable oothecae: 4

Mean number of larvae hatched from one ootheca — 38.3

Days required from start of the rearing to egg hatching — 36 days

Discrimination of sex by antennae

From observation of the mating behavior and from the results of the rearing experiment under darkness, it seems that a sexually mature male does not discriminate

Fig. 1. Wing raising of *Blattella germanica* males by contact their antennae to a head of virgin female of the same species which had been attached to a small glass rod. Two males (left side) are raising their wings, and the other one is just prior to wing raising. Wing raising also occurs by contact only with a single isolated virgin female antenna.

a sexually mature female by his visual sense but by contact or chemical stimuli through his antennae. It was most interesting to watch, when a head isolated from a sexually mature female was attached to a small glass rod, the males responded and raised their wings at the touch of their antennae with the head as if they did with intact females (Fig. 1). Furthermore only an antenna isolated from a sexually mature female showed the same effect of wing raising of the males. On the contrary, an antenna isolated from a male had no such effect of wing raising of the males. This experimental evidence supported that there is a remarkable difference between the antenna of the male and that of the female in discriminating sex by males.

Effect of antennae on copulation

As mentioned earlier, the antennae of the males seem to play an important role for discrimination of sex. The following experiment was carried out to confirm this: if the antennae of the male were removed, whether or not the antennectomized male could copulate with females. Antennae of virgin males and females were amputated with a small dissecting scissor as close to the base as possible. Five antennectomized males or females were paired with five virgin intact females or males, and reared in a glass pot, 11 cm diameter, 7 cm height, at 25°C with 14 hr of illumination per day until the period egg hatching. The number of nymphs hatched, and the number of days required from the start of the rearing to egg hatching were recorded. Results are given in Table 1.

As mentioned in the previous paper (ISHII, 1971), the antennectomized cockroaches were dull in their action. However, as seen in Table 1 the antennectomized adults

Table 1. EFFECT OF AMPUTATION OF ANTENNAE ON MATING AND REPRODUCTION OF THE GERMAN COCKROACH

Exp.	Male	Female	Replica- tion	Number of oethecae produced	Days required from start of rearing to egg hatching (days)	Mean number of nymphs from one oetheca
A	Normal 5	Normal 5	3	5	36.3	38.0
				5	37.4	39.0
				5	34.0	44.6
B	Normal 5	Antennectomized 5	4	5	41.4	39.4
				5	33.2	37.8
				4	37.0	36.0
C	Antennectomized 5	Normal 5	3	5	32.8	38.0
				3	37.7	40.3
				3	35.7	41.0
D	Antennectomized 5	Antennectomized 5	3	4	35.0	44.5
				2	52.0	40.5
				2	42.0	31.5
E	0	Normal 5	3	3	46.7	42.7
				0		0
				0		0

were still able to copulate, but the effect of amputation of the male antennae on copulation was more inhibitory than that from females. When antennae of both females and males were deprived, the number of viable oothecae decreased greatly, and the number of days until egg hatching increased. These results indicated that the mating chance decreased by the amputation of the antennae because of dullness in their action. If the same experiment is undertaken in a large rearing container, the copulation seems impossible.

Structure of male and female antennae

The number of anuli of the German cockroach antennae increases with advancing instars, and attains nearly one hundred anuli in the adult as reported in the previous paper (ISHII, 1971).

Observations of the antenna under a stereo scanning electron microscope, JSM-

Fig. 2. Fine structure of the anulus of the German cockroach antennae. All the anuli shown are nearly tip of the antennae ($\times 700$).

A: Female adult, B: Male adult, C: 6th instar nymph of female, D: 6th instar nymph of male.

U3, Japan Eletron Optics Laboratory Co., Ltd., showed that there are three types of hairs distributed on the cuticle of the anuli, and these three types of hairs are about the same as those of the 1st instar nymph except for increasing size, number and distribution.

There are no remarkable differences in the kinds of hair and their distribution between sexes and between adults and that of the 6th instar nymph as seen in Fig. 2. From results of the stereo scanning electron microscopic observation, it seems likely that physical factors of the female antenna do not play an important part in the discrimination of sexes by the males.

Wing raising potency of the legs and wing of the female

From the results of rearing experiments with antennectomized females, given in Table 1, it seemed that parts other than the antennae of the female prompt the wing raising of males. Fore wings, fore legs, mid legs, and hind legs isolated from virgin females were tested with sexually mature virgin males. The results are given in Table 2.

Wings and legs isolated from sexually mature virgin females have the potency for wing raising of males, but this potency appeared less than that of the antennae isolated from females. The fact that the wings and legs isolated from the females have potency suggests the structure of antennae is not so important for wing raising, but some chemical factors seem to play an important role for it.

Wing raising potency of antennae isolated from 6th instar nymphs

The wing raising activity of antennae isolated from both males and females of the 6th instar nymphs were tested with sexually mature virgin males. Results are given in Table 3.

It is of interest to note that antennae from males of the 6th instar nymphs have the wing raising potency although the potency is less than those from the females. As seen in Fig. 1, there was no remarkable difference in the structure of antennae between female and male of the 6th instar nymphs.

Table 2. WING RAISING POTENCY OF WINGS AND LEGS ISOLATED FROM SEXUALLY MATURED FEMALES

Isolated organ	No. of test females	Per cent male responded ^a
Left fore-wing	4	54.2±14.4%
Right fore-leg	4	64.6±21.9
Right mid-leg	4	67.7±13.7
Right hind-leg	4	61.5± 7.0

^a Each leg or wing tested with 24 sexually matured virgin males.

Table 3. WING RAISING POTENCY OF ANTENNAE ISOLATED FROM THE 6TH INSTAR NYMPHS

Sex	No. of antennae tested	Per cent male responded ^a
Female	11	90.5±13.9%
Male	10	57.1±30.4

^a Each antenna tested with 24 sexually matured virgin males.

Fig. 3. Change of wing raising potency of male antennae after adult emergence. Numbers given in this figure indicate number of antennae tested, and each antennae was tested against 24 German cockroach male adults. Each percentage shows mean value with 95% fiducial limit.

Effect of adult age on wing raising

UEDA *et al.* (1969) reported that female and male adults of the German cockroach matured sexually about one week after adult emergence, and thereafter they copulate. Antennae isolated from both females and males in different ages of adults were tested with sexually mature males. Results are shown in Figs. 3 and 4.

As seen in Fig. 3, antennae isolated from newly emerged male adults had wing raising potency to a certain extent, but the potency decreased with the advance of days and then lost its potency completely after 5 days. The potency was not recovered until termination of the experiment. On the contrary, the wing raising potency of antennae isolated from newly emerged female adults was small and fluctuated among individuals, but it increased rapidly with advance of days and attained almost completely after 9 days (Fig. 4). The potency was not decreased until 25 days.

DISCUSSION

The sequence of courtship of the German cockroach has been observed by ROTH and WILLIS (1952), and they found that sex discrimination by males is mainly due to contact chemoreception by their antennae. The results of the present experiments confirmed their observations. The following evidences support this conclusion:

(1) There was no remarkable difference in structure of antennae between male and female adults, and between adult and the 6th instar nymph.

Fig. 4. Change of wing raising potency of female antennae after adult emergence. Numbers given in this figure indicate number of antennae tested, and each antennae was tested against 24 German cockroach male adults. Each percentage shows mean value with 95% fiducial limit.

(2) Antennae isolated from 6th instar nymphs of both male and female have wing raising potency to males.

(3) Wings and legs isolated from sexually mature virgin females also have the wing raising potency to males.

(4) Antennae isolated from sexually immature females showed less wing raising activity to males than those from sexually mature females. Antennae isolated from sexually immature males showed wing raising activity to a certain extent.

These experimental results supported that some chemical substance(s), localized mainly in the female antenna, plays an important role for discriminating sexes. And the sexually mature male can discriminate females by touch with their antennae. The chemical factor(s) responsible for the wing raising of males is not only localized in the sexually mature female antennae but also in other external organs such as the wings and legs. This was also confirmed by the fact that antennectomized females were still able to copulate with males as shown in Table 2. The antennectomized insects are dull in their action so that if they are reared in a larger container than the present one used, chances to meet and copulate will be decreased.

Physical factor(s) of sexually mature female antennae seems to play some role for wing raising of males to a certain extent but it must be small. If some chemical factor(s) of sexually matured female antennae plays an important role for wing raising of males, there are the following two possibilities:

- (1) Sexually mature females possess some chemical factor(s) responsible for wing raising of males in their cuticular surface, especially in the antennae — female factor.
- (2) Sexually mature males have some chemical factor(s) responsible for the inhibition of the wing raising factor in their cuticular surface — male factor.

The facts that the antenna isolated from newly emerged female adults is poor in the activity but increases rapidly and attains complete response from males after about 10 days, and that the activity is lost when the antenna are washed with organic solvents such as ether and *n*-hexane (unpublished data), suggests the presence of a “female factor” in the cuticle of females.

On the other hand, the fact that the antennae isolated from newly emerged adult males has the activity to some extent, but the activity is lost with progressing age, suggests that a “male factor” is contained in the cuticle of sexually mature males. It is still difficult to conclude that the wing raising of males is regulated by either the “female factor” or the “male factor”, but it seems the “female factor” contained in the cuticle of sexually mature female antennae has to greater attention.

Chemical nature of the “female factor” is now being studied with several chemical reagents and biological tests, and it will be published in a paper to follow. If the “female factor” could be characterized, it will be considered as a kind of sex pheromone responded by contact chemoreception of the male antenna.

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