The Nature and Development of Sex Attractant Specificity in Cockroaches of the Genus *Periplaneta*

III. NORMAL INTRA- AND INTERSPECIFIC BEHAVIORAL RESPONSES AND RESPONSES OF INSECTS WITH JUVENILE HORMONE-ALTERED ANTENNAE

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ABSTRACT Male cockroaches of the species *Periplaneta americana* rely on a female-produced airborne pheromone to initiate courting behavior. The male responds to the presence of the pheromone with rapid, oriented locomotion. Contact with the female (or other males or larvae) elicits wing-raising and other responses characteristic of male courtship behavior. Males of other species within the genus *Periplaneta* also respond to a female-produced pheromone, but with less intensity than *P. americana.* Cross-species testing shows that *P. brunnea* males respond to the *Periplaneta* pheromone with the same intensity elicited by the *P. brunnea* pheromone. A second reaction group containing *P. australasiae, P. fuliginosa,* and *P. japonica* also responds to the *P. americana* pheromone and their conspecific pheromones, but with a low intensity characteristic of these species. Alteration of the antennal morphology of *P. americana* males can be experimentally induced by manipulating the level of juvenile hormone during development. Males with a full adult complement of olfactory receptors all respond behaviorally to the pheromone. Adult males with larval antennae produced by bilateral treatment with exogenous juvenile hormone-mimic do not respond to the pheromone, although they are completely adult in other respects.

Cockroaches employ pheromones or sex attractants to initiate courtship behavior (Roth and Barth, '67). The mating behavior of species within the genus *Periplaneta,* especially that of the American cockroach, *Periplaneta americana,* has been described in detail by several workers (Roth and Willis, '52; Wharton et al., '54a,b; Barth, '61, '70; Roth and Barth, '67; Frazier, '70; Simon, '71). In general, these studies indicate that in the genus *Periplaneta* the initial step in male mating behavior is olfactory reception of a female-produced sex attractant.

Mating behavior in cockroaches is also under endocrine control. The production of sex attractant by adult females in some species is under the control of the corpora allata, endocrine glands associated with the cerebral ganglia (Barth, '65, '68). In *P. americana* sex attractant is produced in large quantities by virgin females one to two weeks after the adult ecdysis (Wharton and Wharton, '57; Bodenstein, '70). Pheromone production stops during pregnancy and generally declines with age (Barth, '68). The midgut is the most likely site of pheromone production in *P. americana* (Bodenstein, '70), which makes female feces a good source of sex attractant for experimental studies (cf. Raisbeck, '75).

Sex attractant reception in males is accomplished by antennae which have a large number of olfactory sense organs (Roth and Barth, '67). As in most insect an-

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tennae (Slifer, '68), the olfactory sense organs consist of porous, thin-walled hairs containing the dendrites of sensory neurons. Sexual dimorphism is clearly evident in the antennae of *P. americana*, in that the density and total number of olfactory hairs in adult males is nearly twice that of adult females, although the antennae are the same size (Schafer and Sanchez, '73; Lambin, '73). Similar sexual dimorphism is found in antennae of other cockroaches of the genus *Periplaneta* (Schafer and Sanchez, '76a).

Experimental alteration of juvenile hormone (JH) titer in developing *P. americana* demonstrates that the near-doubling of male olfactory sensilla at the terminal ecdysis is regulated by JH (Schafer and Sanchez, '76b). During larval development, JH inhibits the differentiation of the extra complement of olfactory sensilla characteristic of the adult male. Some olfactory receptors which appear on male antennae at the adult stage are receptors attuned to the female-produced sex attractant (Schafer, '77).

The behavioral experiments reported here further confirm the description of natural sexual behavior in *Periplaneta* spp. (Barth, '70; Simon, '71), and support the observations of Frazier ('70) and Simon ('71) on interspecific responses to sex attractants within the genus *Periplaneta*. Of physiological interest are the behavioral responses of larval and adult *P. americana* whose antennae have been morphologically altered by experimental modification of JH activity during postembryonic development. For example, the differentiation of male antennal sensilla can be inhibited by dipping the antennae in a solution containing juvenile hormone-mimic (JH-M) during the terminal larval stage (eleventh instar). This treatment results in the emergence of normal-appearing males with larvae-like antennae (Schafer and Sanchez, '76b). Both unilateral and bilateral antennal dips depress olfactory sensillar development in the antennae exposed to JH-M. Since sex attractant receptors are specifically inhibited by this treatment (Schafer, '77), it is of interest to examine the treated animals' behavioral responses to the sex attractant.

MATERIALS AND METHODS

Experimental animals

The *P. americana* (L.) used in this study were obtained from several sources and showed some differences in size and activity, as well as statistically supportable differences in the distribution of antennal sense organs (Schafer and Sanchez, '76a). The designations of the various strains of *P. americana* and their sources are as follows: *P.a.*/MR from P. W. Winston at the University of Colorado, Boulder; *P.a.*/BE from V. Adler and O. Bodenstein at the U.S.D.A., Beltsville, Maryland; *P.a.*/UR from J. G. Sternburg at the University of Illinois, Urbana-Champaign; and *P.a.*/WE, a strain with white eyes, from F. W. Fisk at the Ohio State University, Columbus. *P.a.*/UR were the largest and most active insects. *P.a.*/UR were used for most of the experiments on normal animals, but it was necessary to use insects from a larger stock culture of *P.a.*/MR for the hormonal experiments. Virgin adult males and females were tested within one to four weeks of emergence from the terminal ecdysis. Terminal instar larvae (eleventh instar) were tested one to two weeks after emergence from the last larval ecdysis.

Other species within the genus *Periplaneta* were obtained from the following sources: *P. japonica* from D. Alsop at Queens College of the City University of New York, Flushing; and *P. australasiae* (Fabricius), *P. brunnea* Burmeister, and *P. fuliginosa* (Serville) from F. W. Fisk.

Pheromone sources

Crude extracts containing *P. americana* sex attractant were prepared from the feces of adult virgin females one to five weeks post-emergence. Control extracts were prepared from the feces of virgin males and the combined feces of male and female larvae. Thus, three types of extract

were available for testing: female extract (containing some fecal odors and sex attractant), male extract (containing some fecal odors and any male-produced pheromones), and larval extract (containing some fecal odors and any larva-produced pheromones) (cf. Roth and Cohen, '73; Bell et al., '73; Bell et al., '72). *P.a.*/UR and *P.a.*/BE were used for all fecal collections.

Extracts were prepared by grinding 1.0 g of feces in 10 ml dichloromethane-methanol (3:1), filtering, and rinsing the residue in the filter with four 10 ml volumes of dichloromethane-methanol. The extract was then dried *in vacuo*, and the dry residue ground in 4 ml purified n-hexane and filtered. The filter was rapidly washed with four 2 ml volumes of n-hexane and the filtrate stored until use in tightly-capped and individually isolated bottles. Based on behavioral and electrophysiological tests, such extracts remained fully active for more than a year.

Highly purified *P. americana* sex attractant was supplied by K. Nakanishi and J. Tabak of the Department of Chemistry, Columbia University. The pheromone supplied by Nakanishi and Tabak was dissolved in purified, spectral grade n-pentane and was a one hundred-fold dilution of the stock solution held at Columbia. Tabak had previously determined that substantial behavioral activity remained after a one million-fold dulution of the stock solution. Although the pheromone supplied probably represented a single molecular species (purified from the more active of two attractive fractions), the chemical identity and the exact concentration were unknown.

Live and killed insects (virgin females, virgin males, and larvae) were also used as odor sources. Live insects were placed in small ventilated cages six to eight hours before testing. Dead insects were killed with CO_2 30 minutes before testing and placed in cages. The prepared cages were presented during testing in the same manner as the crude extracts and purified pheromone.

Testing conditions

The insects were entrained to a 10-hour photoperiod (10L/14D) and tested under dim red light two to six hours after the onset of darkness. Insects were isolated four to eight days prior to testing in groups of five in separate 10-gallon glass aquaria 25 × 50 cm × 30 cm h. Each aquarium was covered with fitted Plexiglas top with a hole bored in the center to permit introduction of odorous stimuli. Three sides and the bottom of each aquarium were covered on the outside with white paper ruled with two crossed black lines dividing each surface into four equal rectangles. The transparent front and top were similarly ruled.

For testing, a single 4 cm^2 filter paper (Whatman no. 1), impregnated with fecal extract or purified pheromone, was pinned to the bottom of a rubber or polyethylene stopper. The stopper was then inserted in the hole. In a typical experiment, presentation of the pheromone-containing filter paper was preceded by 5-minute tests with each of the following controls: a blank filter paper, filter paper with larval extract, and filter paper with male extract. Filter papers impregnated with 0.01, 0.1, 0.2, 0.4, or 1.0 ml of fecal extract were used, with 0.1 ml used routinely in most tests. In tests with the purified pheromone, filter papers with 0.001, 0.01, or 0.1 ml were used, with blank and pentane-treated filter papers presented first as controls.

The activity of a single, marked cockroach among the group of five in the aquarium was scored by counting the number of times it crossed any ruled line or changed surfaces. The number of crossings in the first, second, and third minutes after introduction of the pheromone source was counted as *activity counts per minute* (ACPM) after the method of Block and Bell ('74) and Bell et al. ('74). The latency between the presentation of the odor source and the first movement of the marked insect was also recorded. All five insects were observed for any instances of

male sexual behavior (partial wing-raising; full wing raising display, with turning; and backing and copulatory thrusts), or pseudofemale behavior (mounting and feeding on the backs of displaying males). Any other types of behavior which occurred were also recorded, e.g., aggressive activities such as stilt-walking, leg and abdomen shaking, and quick wing flicks.

Categories of insects tested

Adult males, adult females, and terminal instar larvae (eleventh instar) of the species *P. americana* were tested using crude extracts, purified pheromone, and live and killed insects as odor sources. *P.a.*/UR were used most often. Adult males of four other *Periplaneta* species were also tested using the *P. americana* extracts and purified pheromone. However, they were *not* extensively tested against extracts of feces from females of their own species because of inadequate numbers of insects for fecal collections. Males of all *Periplaneta* species were tested against confined live females of their own species.

Tests of the hormonally-altered *P. americana* were performed using fecal extracts. Purified pheromone was not available in sufficient quantity to apply in these tests. Consult Schafer and Sanchez ('76b) for the morphological effects of hormonal alterations and the rationale behind each operation.

RESULTS AND DISCUSSION

Behavior of Periplaneta americana

The courtship behavior of *P. americana* has been described by a series of workers who were initially interested in the role of the airborne pheromone (Roth and Willis,-'52; Wharton et al., '54a,b), but went on to produce complete ethological descriptions of courtship and mating (Barth, '61, '70; Simon, '71; Rust et al., '76). The general pattern of *P. americana*'s mating behavior has been admirably condensed in the ethogram published by Barth ('70; p. 730). Behavior under the conditions of these tests is as follows:

1. *Response of male P.a. to a receptive female*

The male is initially motionless, but within 10 to 40 seconds increases the frequency of antennal waving and raises the head and anterior part of the body (*attentive posture* of Barth, '70). The male begins to move about rapidly, usually 10 to 15 seconds after assuming the raised posture. The movement is often directed toward the female (cf. Rust and Bell, '76) and may be accompanied by partial wing raising when encountering another male or larva. The male then touches the female with its antennae and initiates a full wing raising display. Turning and backing movements accompany the display and result in a tail-to-head relationship of male and female (*presentation* by the male). Mounting of the male by the female releases or is accompanied by backing up and abdominal thrusts by the male. The male's wings are fully spread (the *dragon-fly posture* of Barth, '70) while the female mounts. Copulation may follow after genital connection, but usually the female flees (*decamps*, Barth, '70) either after presentation by the male or after mounting the male and using the palps to examine the dorsal thorax of the male. The area examined corresponds to the location of the tergal gland found in other species of cockroaches, although no well defined tergal gland is apparent in *P. americana*. The many variations on this general theme and the roles of probable releasers have been described by Barth ('61, '70).

2. *Response of male P.a. to a confined female*

The male's response to a live or killed female confined to a perforated container is identical to the response to a free female up to the point of location of the container by the male. Antennal contact with the container (or a part of the female protruding through a perforation) elicits wing raising and intense palpal exploration of the container surface. Turning and backing movements are always seen, but they are

disoriented or incomplete. The male cockroach may continue to examine the container for many minutes and show repeated wing-raising displays.

3. *Responses of P.a. males to control extracts*

In all experiments using fecal extracts containing sex attractant, the insects were first presented with controls consisting of larval and male extracts. In about 10% of the control presentations an attentive posture was elicited in one or more of the five males in the behavioral arena. In no case was activity increased more than four activity counts per minute (ACPM) over the background level. Wharton et al. ('57) reported that larval females sometimes produce attractant, but the larval extract used in the present study was no more effective in eliciting increased activity than the male extract. In a few instances, usually involving larvae, an insect would actively seek and locate the source of the control odor. However, the explosive activity increase associated with introduction of the sex attractant extract (see below) was never observed with control extracts, nor was male display activity elicited by control stimuli. The introduction of food odors (banana, apple, and pear) had some effect on the activity of well-fed cockroaches. For example, introduction of banana odor to insects raised on apples and lab chow elicited an average activity of 7 ACPM. In deprived cockroaches, food odors induced increases in both activity and aggression (see below).

4. *Responses of P.a. larvae and adult females*

Normal *P. americana* females and eleventh instar male larvae were tested with fecal extracts. In two trials apiece with 60 adult females and 60 male larvae, no definite and repeatable response to the sex attractant could be detected. An attentive posture and slightly increased activity (4-5 ACPM) were observed in about 15% of the male larvae presented with the sex attractant. This low level response could represent a genuine response to the pheromone, since it was not elicited as readily using larval and male extracts.

5. *Background activity*

The background activity level observed during the active portion of the diurnal cycle ranged from 0-3 ACPM. Adult males were always more active than adult females or larvae. In groups of isolated males, "spontaneous" homosexual courtship was sometimes seen during the active period of the diurnal cycle. These displays were always elicited by contact between insects and tended to involve only one or two active males. Sexual displays by one male to another were typically met with aggressive responses by the courted male. The level of "spontaneous" sexual displays increased with the duration of the isolation period, but was never high enough to interfere with tests using fecal extracts or purified pheromone.

6. *Response of male P.a. to pheromone*

As with the introduction of a live female, the male is initially motionless when a filter paper containing female fecal extract or purified pheromone is inserted. After a latency of 10 to 40 seconds, antennal waving increases, and the anterior part of the body is raised. The antennae are often held in a nearly vertical position. Rapid movement ensues five to ten seconds after assuming the raised, or attentive, posture. The increase in activity among a group of isolated males is explosive and is relayed to the other males in the arena by the first male responding to the pheromone. A male first runs about the floor of the behavioral arena, then moves up onto the walls, and finally onto the ceiling of the container where it may locate the pheromone source. These movements are usually zig-zag, but occasionally the roach will move directly up a wall and across the ceiling to the pheromone source. While moving about the floor, walls, or ceiling of the arena, the male may display partial wing-raising

which is usually correlated with contacting another insect. Some males may present and display the dragon-fly posture to other adult males or larvae, or occasionally to inanimate objects, such as pieces of lab chow. Homosexual or pseudo-female activity may occur in the form of mounting a displaying and presenting male. When the pheromone-containing filter paper has been located, the male produces a partial or full wing-raising display within a second after the palps or antennae touch the filter paper. Intense palpal examination of the filter paper accompanied by repeated wing-raising may occur, or the insect may simply resume its rapid movement about the arena. The intense wing-raising elicited by contact with the filter paper often declines rapidly even though the insect remains near the paper. This response is most aptly described as calming. Courtship activity may go on for more than an hour if the pheromone source is left in the arena.

Quantification of the response of normal males to 0.1 ml of fecal extract is presented in table 1. These data are typical of the results seen in 400 observation sessions involving 2,000 insects over a period of two years using variations of the method reported here. The average time from the introduction of the pheromone source to the assumption of the attentive posture was 35 seconds. This latent period is presumably the time required for diffusion of the pheromone from the filter paper to attain a threshold concentration in the vicinity of the insect on the floor of the arena. Movement was always very rapid, with an average of 76 ACPM in *P.a.*/UR, the most responsive strain. Similar activity levels were elicited by the presentation of caged virgin females, alive or freshly-killed. The use of more fecal extract (0.2, 0.4, and 1.0. ml) did not increase the activity level or display frequency by more than 25%, indicating that the level seen in table 1 is near maximal under the conditions of this assay. Use of the higher pheromone concentrations lessened the number of insects successfully locating the pheromone source. This can reasonably be attributed to the rapid saturation of the air in the chamber with the pheromone and the resulting loss of a pheromone concentration gradient. Use of 0.01-0.1 ml of purified pheromone also produced levels of activity and display similar to that produced by 0.1 ml of fecal extract. With 0.1 ml of the purified pheromone or 0.2-1.0 ml of fecal extract, longer-lasting periods of activity and display were observed, although the initial level of activity was no more than 25% above the average observed with 0.1 ml of fecal extract.

The major factors influencing response levels other than pheromone concentration were (1) time of day, (2) time of isolation of the male from females, and (3) the number of other male cockroaches or larvae in the behavioral arena. These factors were not systematically studied, but several points were obvious. Males were most responsive during the most active period of their diurnal cycle; hence they were tested two to six hours after dark. Responsiveness to the pheromone was low immediately after being placed in the behavioral arena (1-2 days), but rose steadily and reached a plateau seven to ten days after isolation. Insects were not tested beyond two weeks of isolation in order to prevent the development of hypersensitivity and possible responses to non-pheromonal odorants (cf. Bowers and Bodenstein, '71). Activity levels (ACPM) were enhanced by testing insects in groups as demonstrated by Wharton et al. ('54a). Single, isolated males responded with oriented locomotion to the pheromone, but unreliably and at a level substantially below that of a male tested in the presence of four other male adults or larvae. Wing raising displays were not seen in single males until the point at which the filter paper was located and touched.

In addition to the courtship activities listed above, an increase in aggression was invariably associated with the presence of pheromone, particularly after the initial high activity level had subsided or when low levels of pheromone were used (e.g., 0.01 ml of female fecal extract). The activities interpreted as aggression were

jerks or thrusts of the whole body, shaking of a metathoracic leg, lateral abdominal oscillation (2 or 3 twitches from side to side), leg extension, stilt-walking, and quick vertical wing flicks (*wing fluttering* or *wing flashing* of Simon, '71). Reciprocal lateral abdominal oscillation was often seen between pairs of insects during agonistic encounters. Most of these activities were also observed between adult males in the absence of pheromone, between adult females, between adult males and females, between adults and larvae, and between larvae. Biting by aggressive females was also observed. The frequency of aggressive acts elicited in the presence of pheromone was greatest in male-male encounters as observed by Bell and Sams ('73). Aggression was also high between isolated adult females, but it could not be determined if the sex attractant enhanced female aggression (cf. Simon, '71).

It would be unwarranted to attribute increased male aggressiveness to a direct action of the pheromone, because the increased activity level released by the pheromone resulted in a greatly increased rate of male-male or male-larva encounters, and in fact, most aggressive behavior was seen after an initial burst of activity and display lasting 5 to 15 minutes. There is, however, at least an indirect connection between the presence of the sex attractant and increased aggressive behavior. Sexual motivation correlated with increased aggressiveness has also been observed in stridulating male crickets (Alexander, '61; Morris, '71). The problem is part of the larger ethological question of appeasement versus arousal in sexual behavior (Barlow, '70) and deserves further study in the cockroach with appropriate controls for the effects of tactile and chemical stimuli. In support of a direct action by the pheromone, a few isolated males and females showed stilt-walking and abdominal shaking after contacting or reaching the vicinity of the pheromone-impregnated filter paper in the absence of contact with other insects. Territoriality was also observed and may have played a role in aggression, particularly among females, but the relationship was not obvious (cf. Ewing, '72, '73; Ewing and Ewing, '73; Bell and Sams, '73).

Intra- and interspecific responses to the P.a. sex attractant

Quantitative differences were noted in the responses of males of different strains of *P. americana*, specifically the intensity and duration of activity and display. There were no qualitative differences between strains, such as the complete omission of a given display. By far, *P.a.*/UR was the most responsive strain, even though the numerical data of table 1 do not adequately convey the intensity of the response apparent in viewing the actual behavior. In a pilot study, fecal extracts of females of each strain were prepared and tested against the strain of origin. These tests, and additional tests using five females, indicated that the observed differences in responsiveness were not due to the origin of the sex attractant, but reflected varying levels of responsiveness characteristic of the different strains.

The cross-species tests (table 1) indicate that all the species tested detect the *P. americana* pheromone. Activity in *P. brunnea* is stimulated strongly by the *P. americana* pheromone while the other species respond with only a slight activity increase *(P. australasiae)* or not at all *(p. fuliginosa* and *P. japonica)*. Tests of *P. brunnea* males with fecal extracts from *P. brunnea* females indicated that *P. brunnea* responds as strongly to the *P. americana* pheromone as it does to its own pheromone.

Tests in which live or killed females were used showed that *P. americana* is, by far, the most reactive to the airborne pheromone. *P. brunnea* males also responded well when presented with females of their own species, but less strongly than *P. americana* males to *P. americana* females. Male *P. australasiae, fuliginosa,* and *japonica* were all much less responsive to their own (confined) females than *P. americana* and *brunnea*. Wing-raising, presenting, and pseudo-female behavior were observed in

TABLE 1

Average response of Periplaneta *spp. males to* P. americana *sex attractant*

	Responses of individually-marked insects [1]			Responses of all insects in behavioral arena [2]				
	Number of insects tested [3]	Number of observation sessions	ACPM [4] in first three minutes	Attentive posture	Increased activity	Raised wing display	Presenting	Pseudo-female activities
Species								
P. americana strains								
P.a./MR	20(100)	48	65	100%	100%	100%	58%	48%
P.a./BE	15(75)	35	63	100	100	100	60	54
P.a./UR	30(150)	62	76	100	100	100	82	68
P.a./WE	6(30)	12	81	100	100	100	83	75
P. australasiae	6(30)	14	4	44	20	5	4	4
P. brunnea	20(100)	28	21	100	100	32	25	25
P. fuliginosa	6(30)	14	3	43	0	0	0	0
P. japonica	6(30)	14	2	30	0	0	0	0

[1] These data are taken from observations on a single, marked insect among the group of five present during an experiment.
[2] These data pertain to all five animals present in an experiment. If any one animal showed the listed response, that was counted as a positive response. For example, presenting was observed in 28 out of 48 observation sessions in *P.a.*/MR or in 58% of the sessions (second column from right).
[3] The first number indicates the number of marked insects tested. The number in parentheses indicates the total number of insects observed (in each experiment, the marked insect plus four companions).
[4] ACPM, activity counts per minute.

all species, but the frequency of these acts was low and of much longer latency in *P. australasiae, fuliginosa,* and *japonica.* Displays in *P. brunnea* were never elicited by the pheromone alone; contact between individuals always preceded display.

The data of table 1 should not be taken as directly comparable measures of the interspecific effectiveness of the *P. americana* pheromone. Each species starts from its own intrinsic level of responsiveness to the airborne pheromone. *P. americana* happens to produce the most intensive activity and displays of the five species tested. The results therefore indicate a similarity among the attractant(s) produced within the genus *Periplaneta,* but a variable reliance of the five species on the airborne attractant. It is likely that reproductive isolation among the species of the genus *Periplaneta* does not depend on the existence of chemically unique airborne attractants. Important isolating merchanisms probably come into play when males and females are at very short range, within the mutual influence of each other's vibratory and contact stimuli.

Behavior of hormonally-altered insects

Many of the hormonally-altered insects were sacrificed for morphological examination of the antennae before the need for behavioral tests became clear. Hence, the number of insects tested behaviorally is small in comparison to the number of normal insects tested. In the following discussion, comparisons to normal insects refer to the strain *P.a.*/MR from which the hormonally-altered insects were derived.

1. *Antennectomy experiments*

Eleventh instar larvae subjected to unilateral antennectomy generally ecdyse to an extra, twelfth, instar rather than ecdysing to the adult stage. Male twelfth instar larvae have larval antennae, but adults derived from twelfth instar larvae at a subsequent ecdysis develop the adult pattern (Schafer and Sanchez, '76b). Behavioral tests on three twelfth instar larvae (12 trials) showed no response of any kind to the sex attractant. Twelve trials on three adult males derived from twelfth instar larvae showed adult male behavioral responses: attentive posture, increased ac-

tivity (7-14 ACPM), wing-raising, and presenting. Normal adult responses were also seen in three adult males (in 12 trials) derived from unilaterally antennectomized eleventh instar larvae. Thus, adult sexual behavior was absent in male insects with morphologically larval antennae (twelfth instar larvae), but appeared with the development of the adult pattern of antennal sensilla.

2. *Allatectomy experiments*

Removal of the corpora allata of tenth instar larvae resulted in the appearance of adultoids with the adult pattern of antennal sensilla (Schafer and Sanchez, '76b). Male adultoids responded vigorously to the attractant in all trials (N= 3, 12 trials), showing increased activity (6-25 ACPM), wing-raising displays, and presenting. Female adultoids (N= 3, 28 trials) adopted an attentive posture in about half the trials and showed slightly increased activity in four trials (3-5 ACPM). Female adultoids generally displayed more aggressive behavior (biting, stilt-walking, abdominal shaking, etc.) than normal females. I gained the impression that the introduction of pheromone or a live (but confined) female increased the level of aggressive acts. Usually though, such an increase was not immediately observed and required 5 to 15 minutes to develop.

3. *Juvenile hormone experiments — whole-animal treatment with JH-mimic in the eleventh instar*

Twelfth instar male larvae produced by whole-body exposure to juvenile hormone-mimic (JH-M) were not tested behaviorally. However, male larvoids (N= 2, 6 trials) and semi-adults (N= 4, 8 trials) responded to the pheromone with increased activity (8-15 ACPM). The semi-adults, which have wings, showed no raised wing displays or presenting. Thus larvoid and semi-adult males possessing adult-like antennae (Schafer and Sanchez, '76b) responded to the pheromone with activity, but not with displays normally seen in adult males. Adult males derived from twelfth instart larvae were not tested.

4. *Juvenile hormone experiments — treatment of antennae with JH-M in the eleventh instar*

All insects tested behaviorally received a 60-second dip in 70% ethanol containing JH-M, concentration 1:100, in the early part of the eleventh instar (Schafer and Sanchez, '76b).

Bilaterally-treated insects. Sixty-five percent (N= 20, 66 trials) of male adults derived from bilaterally-treated eleventh instar larvae showed absolutely no response to the sex attractant. The remainder of the bilaterally-treated insects (35%) responded to the sex attractant with a variable amount of increased activity (average, 15 ACPM). Approximately one-third of the 35% which responded to the pheromone showed wing-raising or other sexual displays. Thus, bilateral treatment with JH-M inhibited sexual responses in the majority of insects treated. No inhibition was observed in control animals bilaterally treated with 70% ethanol. Insects treated bilaterally with JH-M responded to food odors at the same level as normal insects: weakly when well-fed; strongly when starved.

Unilaterally-treated insects. All insects which received unilateral JH-M treatment responded to the sex attractant with the normal, explosive activity increase (N= 10, 25 trials; average ACPM= 30). Raised wing display, presenting, and pseudo-female activity were at about half the level of normal males. Thus, unilateral treatment with JH-M measurably depressed display responses. Nevertheless, *all* unilaterally-treated insects responded with increased activity which contrasts with bilaterally-treated insects where the majority showed no response of any kind.

Significance of the hormonal experiments

Receptors which respond to the sex attractant account for a portion of the extra olfactory sensilla which appear on male *P. americana* antennae at the terminal ecdysis (Schafer, '77). In these experiments, every male with a morphologically adult

antenna responded behaviorally to the pheromone, including the larvoids and semi-adults which have a number of larval characteristics, but adult-like antennae. Thus, the development of the adult pattern of peripheral receptors is accompanied by male sexual behavior.

The determinants of central circuits which program sexual behavior remain unexplored. Since all male *P. americana* with adult-like antennae responded behaviorally to the attractant, we know that *both* the peripheral receptors for the attractant *and* the central circuits necessary to the behavior were present. This fact could have one of several corollaries: (1) the differentiation of peripheral attractant receptors and the activation of the appropriate central circuits depend upon similar JH concentrations; or, (2) the differentiation of the peripheral receptors depends on JH, but the central circuits are already in place waiting for the arrival of the appropriate input; or (3) the differentiation of the peripheral receptors depends on JH, and the activation of the central circuits depends on a trophic effect produced by the ingrowth of receptor axons. The present experiments cannot be used to choose among these three possibilities. Because there are a number of examples of hormonally-programmed insect behaviors (e.g., Milburn et al., '60; Loher and Huber, '66; Truman and Sokolove, '72), it may be that the first possibility is the most likely.

What determines male versus female sexual behavior in a given insect beyond the presence or absence of sex attractant receptors? Gynandromorphs of the cockroach, *Byrsotria fumigata,* exist which show both male and female sexual behavior within the same individual (Barth and Bell, '71). *P. americana* males — both adults and larvae — often mount presenting males as the receptive female normally does. This indicates that at least one "female" behavioral circuit exists in larval and adult male brains. The antennal input pathways of the *Periplaneta* brain have been mapped (Boeckh et al., '75), but the precise central connections of sex attractant receptors are unknown. Moreover, such circuits cannot be easily traced beyond the input stage because synaptic connections to second and higher order neurons occur in the neuropil, and its organization is refractory to existing mapping techniques.

The identity of central neural activators or suppressors of sexual behavior in cockroaches is as yet unknown. While JH is a likely candidate for suppressing sexual behavior in larval brains, still other factors are probably at work. For example, Simon ('71) reported that male *P. americana* do not begin to produce behavioral responses to the sex attractant until about five days after emergence from the terminal ecdysis. This lag may be caused by a maturation process in the neural circuitry, or it may be the result of active suppression. Female receptivity declines during pregnancy because pheromone production ceases and the insect becomes more resistant to male courtship. Male and female sexual behavior is therefore plastic; it varies with time and circumstance. A first step in understanding the genesis of the male response has been made in the JH experiments on receptors (Schafer and Sanchez, '76b; Schafer, '77). A second step would be to determine how a differential activation of neural circuits is achieved in development to produce behavioral responses appropriate to male or female.

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