

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

II. JUVENILE HORMONE REGULATES SEXUAL DIMORPHISM IN THE DISTRIBUTION OF ANTENNAL OLFACTORY RECEPTORS

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ABSTRACT Sexual dimorphism of antennal sense organs appears only at the adult stage during normal development of the cockroach, *Periplaneta americana*. Adult males acquire approximately twice as many olfactory sensilla as females at the terminal ecdysis. When terminal instar larvae are subjected to unilateral antennectomy, most ecdyse to supernumerary larvae rather than adults. Sexual dimorphism is not evident in the intact (unamputated) antenna during the extra larval stage, but appears at the following ecdysis which leads to the adult stage. Allatectomy of male and female larvae in the penultimate instar produces adultoids which show antennal sexual dimorphism. Whole-body treatment of terminal instar larvae with exogenous juvenile hormone-mimic (JH-M) results in supernumerary larvae which lack antennal sexual dimorphism. When these superlarvae are removed from the influence of JH-M, they ecdyse to adults with antennal sexual dimorphism. Topical application of JH-M to male antennae early in the terminal larval instar results in the emergence of adults which lack the total male complement of antennal sensilla, but are otherwise normal-appearing. These results indicate that an inhibitory action of juvenile hormone prevents the appearance of antennal sexual dimorphism during normal larval development.

The postembryonic development of antennal olfactory receptors in cockroaches is regulated, in part, by the juvenile hormone (JH) (Schafer and Sanchez, '74). In the African woodroach, *Leucophaea maderae*, the density of antennal olfactory sensilla remains constant at about 400 sensilla per mm² during each stage of larval development, because olfactory sensilla are added at each ecdysis in direct proportion to the increase in antennal surface area. However, at the terminal ecdysis leading to the adult stage, the density of olfactory receptor organs increases to 620 sensilla per mm² in both males and females. Exogenously applied juvenile hormone-mimic (JH-M) prevents this increase; or, the increase can be induced precociously by removing the corpora allata, endocrine organs which produce juvenile hormone in larval insects (Schafer and Sanchez, '74).

We chose to perform similar experiments on the American cockroach, *Periplaneta americana*, since the antennae show distinct sexual dimorphism related to the pheromone-dependent sexual behavior of males of this species. For example, the initial step in courtship behavior of *P. americana* is the male's reception of a female-produced sex attractant (Roth and Willis, '52; Barth, '70; Simon, '71). Of particular interest was the possibility that the differentiation of sex attractant receptors is inhibited by juvenile hormone during larval development. There are approximately twice as many olfactory sensilla on adult male *P. americana* antennae as on adult female antennae (Schafer and Sanchez, '73). In contrast, no sexual dimorphism is seen in the antennae of *Leucophaea* at any

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ANTENNECTOMY EXPERIMENTS

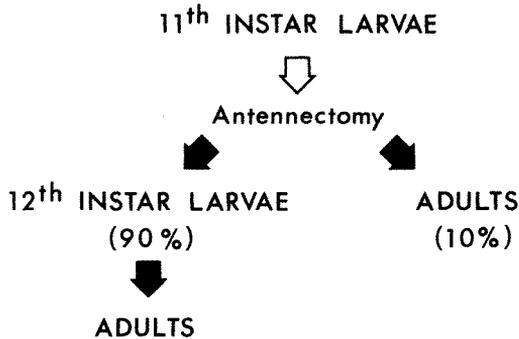


Fig. 1 Protocol for the antennectomy experiments. Eleventh instar larvae were antennectomized on one side only early in the instar. Most ecdysed to supernumerary twelfth instar larvae which subsequently ecdysed to the adult stage. The developing pattern of sensilla was examined in the intact antenna, not the regenerating antenna.

stage of postembryonic development (Schafer, '73). Sexual dimorphism of adult antennae is also characteristic of four other species within the genus *Periplaneta* and in the closely related species, *Blatta orientalis*, but not in *Blaberus craniifer* or many other species of cockroaches (Schafer and Sanchez, '76; Lambin, '73; Schafer, unpublished observations).

MATERIALS AND METHODS

The histological procedures employed in this study have been described fully in the first paper in this series (Schafer and Sanchez, '76). These methods produce transparent whole mounts of newly-ecdysed antennae or older, tanned antennae, and allow direct counting of antennal sensilla. In the work reported here, the total numbers of olfactory and contact chemoreceptor sensilla were extrapolated from direct counts on 20-30% of the flagellar segments on each antenna examined. Density figures were calculated from these data and from measurements of antennal surface area (Schafer and Sanchez, '76).

Experimental animals were maintained at 23°C with ample space and fed lab chow and apples. Even though antennal sensilla differences occur among the several strains of *P. americana* (Schafer and Sanchez, '76),

it was necessary to use insects from two different strains in the present study to obtain a sufficient number of experimental animals. The cockroaches used were cultured in this laboratory: strain *P.a./MR* was used for all experiments except those involving topical treatment of antennae with JH-M where strain *P.a./UR* was used. Comparison data for normal insects were taken from the first paper in this series (Schafer and Sanchez, '76). Experimental animals were subjected to alteration of their normal hormonal regime in the following ways:

Antennectomy experiments (fig. 1)

Trauma, such as epidermal damage or amputation of legs or antennae, induces supernumerary larval instars in cockroaches (Engelmann and Lüscher, '56; O'Farrell et al., '56; Schafer, '73). In this study, a single antenna was amputated at the scape-pedicel joint in each of a series of terminal (eleventh instar) larvae approximately two weeks before the adult ecdysis. Ninety percent of the operated insects passed through a supernumerary (twelfth) instar before ecdysing to the adult stage. The sensilla were examined *in the intact antenna* at each stage. Regeneration of the excised antenna was *not* studied, although

ALLATECTOMY EXPERIMENTS

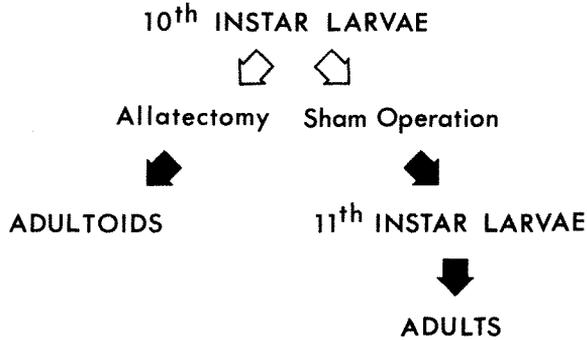
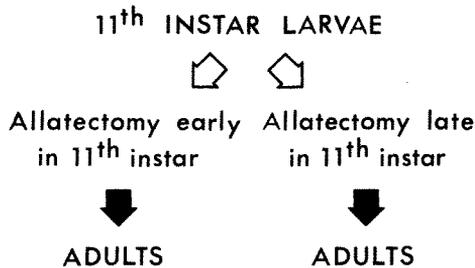
A. ALLATECTOMY in 10th LARVAL INSTARB. ALLATECTOMY in 11th LARVAL INSTAR

Fig. 2 Protocol for the allatectomy experiments.

A The corpora allata of tenth instar larvae were removed early in the instar. These animals ecdysed to adultoids with adult-like morphology. Sham operations were also performed to test the possible effects of surgical trauma. Sham-operated animals ecdysed to normal-appearing eleventh instar larvae and subsequently ecdysed to adults.

B Allatectomies were also performed on eleventh instar larvae as another test of the effects of surgical trauma.

this antenna always regenerated partially if the insect passed through a twelfth instar.

Allatectomy experiments (fig. 2)

The corpora allata were removed from male and female tenth instar larvae, three to seven days after the ninth larval ecdysis. These animals emerged as *adultoids* (Scharrer, '46) about a month after the operation. Corpora allata were also removed from a series of eleventh instar larvae either within three days of the tenth

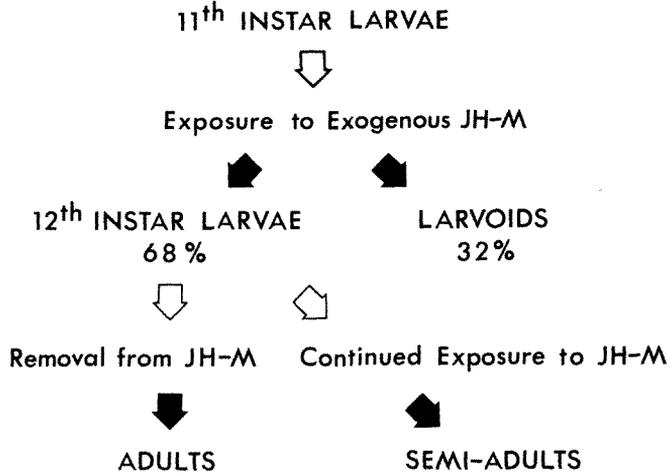
larval ecdysis or late in the instar, about three weeks after the tenth larval ecdysis. Sham allatectomies were performed as controls on tenth and eleventh instar larvae.

Juvenile hormone experiments (fig. 3)

Immediately after the tenth larval ecdysis, groups of male and female larvae were placed in jars containing juvenile hormone-mimic (JH-M). On the floor of each container was a 14 cm disc of Whatman No. 1 filter paper impregnated with 30 mg of

JUVENILE HORMONE EXPERIMENTS

A. WHOLE-ANIMAL EXPOSURE to JH-M



B.-C. TOPICAL TREATMENT of ANTENNAE with JH-M

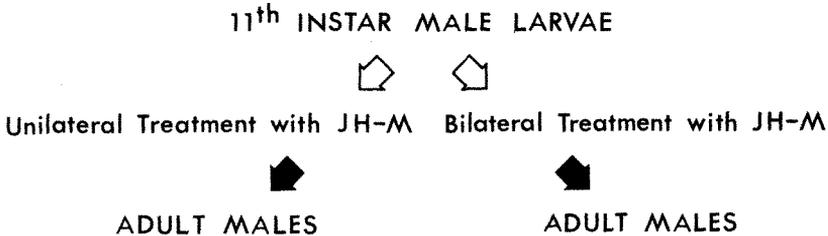


Fig. 3 Protocol for the juvenile hormone experiments:

A Eleventh instar larvae were exposed to synthetic juvenile hormone-mimic. Most ecdysed to normal-appearing twelfth instar superlarvae. Some ecdysed to larvoids characterized by abnormally large wing pads. When twelfth instar larvae were removed from the influence of JH-M, they ecdysed to normal-appearing adults. Twelfth instar larvae left in the presence of JH-M ecdysed to semi-adults characterized by incompletely developed adult features and rumped wings.

B,C Eleventh instar larvae received topical treatment of the antennae with JH-M. One group (B) received bilateral treatment. The other group (C) received treatment with JH-M on one antenna and solvent only on the other antenna.

synthetic JH-M (Calbiochem, synthetic B grade, activity about 1% of that of pure natural JH and at least 100 times more active than dodecyl methyl ether). The JH-M was replenished monthly with an additional 30 mg of hormone. Sixty-eight percent of the insects subjected to this treatment ecdysed to supernumerary larvae (twelfth instar) and had morphological features like those of normal larvae except for

being larger. Thirty-two percent of the treated insects ecdysed to larvae with exceptionally large wing buds like those observed in similar experiments with *Leucophaea* (Shafer and Sanchez, '74) and *Nau-phoeta cinerea* (Radwan and Sehnal, '74). We termed these insects *larvoids* to differentiate them from the completely normal-appearing supernumerary larvae.

Immediately after ecdysis, half the nor-

mal-appearing, twelfth instar, larvae were transferred to a container *lacking* exogenous JH-M. The other half remained in the original JH-M container. All of the twelfth instar larvae removed from the influence of JH-M ecdysed to large, normal-appearing adults. The larvae remaining in the presence of exogenous JH-M ecdysed to adult-like animals with rumped wings. We termed these insects *semi-adults* to distinguish them from normal adults and from normal-appearing adults produced from twelfth instar larvae which were removed from the influence of JH-M. Similar results have previously been obtained with *Leucophaea* (Schafer and Sanchez, '74) and *Blatella germanica* (Das and Gupta, '74). The term *adultoid* will be reserved for precocious adults produced by allatectomy, according to Scharer's ('46) original usage.

A second series of JH experiments was performed in which JH-M was topically applied to only the antennae. The antennae of eleventh instar male and female larvae were dipped in a JH-M solution in an attempt to influence sensillar differentiation. Larvae which ecdysed to adults within two weeks of topical JH-M treatment were discarded on the assumption that the JH-M treatment came after the critical period for JH inhibition. Larvae which ecdysed to adults two to five weeks after the treatment were retained and examined.

Three different concentrations of JH-M were used. One group of males and females received a 60-second antennal dip in 50% ethanol which contained 1 μ l/ml (1:1000) of JH-M (Ayerst Laboratories, Montreal, compound AY-22342). The dip was followed by a 5-minute period during which the animals were restrained to keep them from cleaning their antennae and possibly ingesting the JH-M. The antennae were then washed with 50% ethanol for 15 seconds and the animals freed after another five minutes of restraint. A second group received similar treatment with a higher concentration of JH-M, 10 μ l/ml (1:100) in 70% ethanol, followed by washing in 70% ethanol. A third group received

the same treatment sequence at a still higher dosage of JH-M, 50 μ l/ml (1:20) in 70% ethanol. Unilateral and bilateral JH-M treatments were performed with each concentration. In the unilateral dips, the untreated side was simultaneously dipped in an ethanol solution lacking JH-M. Thus, the untreated antenna served as a control for possible nonspecific effects of ethanol.

Ecdysone experiments

The possible effect of molting hormone on sensillar development (cf. Hangartner and Masner, '73a,b; Masner et al., '75) was tested by treating eleventh instar larvae with ecdysterone. Five micrograms of ecdysterone (Sigma ecdysterone, E-2003) in 10% ethanol were injected into eleventh instar male and female larvae three to ten days after the tenth larval ecdysis. The antennae were examined after the treated animals ecdysed to the adult stage, and compared with the antennae of ethanol-injected controls.

RESULTS

Antennectomy experiments

Ninety percent (N = 50) of eleventh instar larvae which had one antenna removed within two weeks of emergence passed through a supernumerary (twelfth) larval instar before attaining the adult stage. Presumably, the remaining 10% which went directly to the adult stage had passed the critical period for the induction of an additional instar before the antennectomy was performed. Both the twelfth instar larvae and the adults derived from them had normal morphological features except that their body size had increased proportionately.

In the twelfth instar, the total number and density of contact chemoreceptors and olfactory sensilla increased, but no sexual dimorphism developed (table 1B). The increased densities of sensilla might be partially accounted for by a slight drop in the antennal surface area during the twelfth instar, but the absolute number of contact chemoreceptors and olfactory sensilla still increased.

TABLE 1

Normal late development and antennectomy experiments in Periplaneta americana.
All figures are means

	Sex	N	Antennal surface area (mm ²)	Total number of contact chemoreceptors	Density of contact chemoreceptors (/mm ²)	Total number of olfactory sensilla	Density of olfactory sensilla (/mm ²)
A. Normal development (strain P.a./MR)							
Normal tenth instar	M	4	31.4	4,260	136	15,000	478
	F	2	29.2	3,980	136	12,930	443
Normal eleventh instar	M	6	37.8	6,480	171	17,490	462
	F	6	30.6	5,660	185	16,440	538
Normal adult	M	4	41.0	6,640	162	38,910	950
	F	4	37.1	6,410	173	22,070	594
B. Antennectomy experiments (using strain P.a./MR)							
Twelfth instar (from antennectomy in eleventh)	M	3	30.0	7,600	253	18,090	604
	F	3	35.5	7,900	223	20,370	575
Adult (from twelfth instar)	M	3	27.5	11,130	405	32,340	1,178
	F	3	30.7	7,210	235	18,050	588
Adult (from eleventh instar)	M	3	27.6	11,230	407	33,650	1,219
	F	3	28.2	8,430	299	19,250	683

TABLE 2

Effects of allatectomy on antennal development in Periplaneta americana.
All figures are means

	Sex	N	Antennal surface area (mm ²)	Total number of contact chemoreceptors	Density of contact chemoreceptors (/mm ²)	Total number of olfactory sensilla	Density of olfactory sensilla (/mm ²)
A. From allatectomy in the tenth larval instar (using strain P.a./MR)							
Adultoids	M	4	25.4	6,830	269	35,060	1,379
	F	4	18.8	5,600	298	19,140	1,019
Eleventh instar, sham-operated in tenth instar	M	3	22.8	5,560	244	11,870	520
	F	2	23.0	6,590	286	17,420	757
Adults, sham-operated in tenth instar	M	3	26.6	11,360	427	34,580	1,301
	F	3	27.7	8,730	315	21,170	764
B. From allatectomy in the eleventh larval instar (using strain P.a./MR)							
Adult, allatectomy early in eleventh instar	M	3	27.7	10,290	371	31,340	1,131
	F	5	28.8	7,710	268	21,930	762
Adult, allatectomy late in eleventh instar	M	3	27.8	11,970	430	33,110	1,190
	F	3	29.0	6,880	237	20,940	722

Adults derived from twelfth instar larvae showed sexual dimorphism like that of normal adults (table 1B, fig. 4). The number and density of both kinds of sensilla on female antennae was near the level of adults derived from the normal eleventh instar. On male antennae, however, the total number and density of contact chemoreceptors was about twice that of normal male adults. The total number of olfac-

tory sensilla was 83% of normal, but the density was inflated to 124% of normal owing to a decrease in antennal surface area (table 1B).

The 10% of antennectomized larvae which went directly to the adult stage developed sexual dimorphism in both types of sensilla at the normal level. Sexual dimorphism of contact chemoreceptors is characteristic of most strains of *P. ameri-*

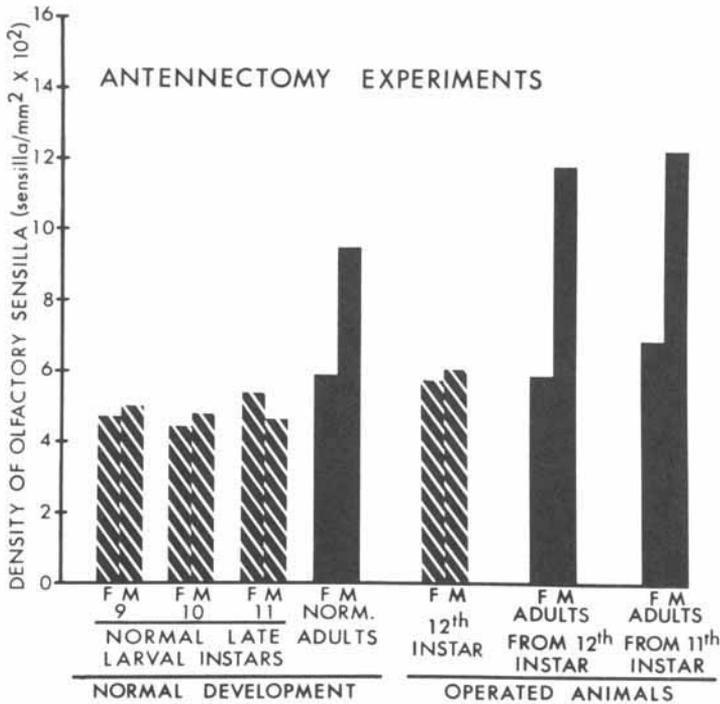


Fig. 4 Results of the antennectomy experiments with respect to the density of olfactory sensilla on the antenna. The experiments were performed as described in the protocol of figure 1. Twelfth instar larvae lacked sexual dimorphism of olfactory sensilla, while adults derived from eleventh and twelfth instar larvae developed sexual dimorphism.

cana, although it normally does not occur in the strain used in this experiment (Schafer and Sanchez, '76). Thus, it may be that experimental manipulation induced the appearance of a latent trait. The alternate explanation is that the original counts on normal animals were faulty. However, reexamination of these antennae and analysis of variance of the counts on them indicate the essential correctness of the original conclusion that sexual dimorphism of contact chemoreceptors does not normally occur in this strain.

Allatectomy experiments

A. Tenth instar larvae (table 2A)

Allatectomy of tenth instar larvae produced adultoids with adult-like morphological characteristics. The antennal surface area in both sexes dropped 20-35% from the level of the tenth instar, but sexual dimorphism developed in both the

total number and the density of olfactory sensilla. Eleventh instar larvae and adults derived from sham-operated tenth instar larvae showed no significant departure from the normal pattern other than a small decrease in antennal surface area which resulted in inflated density figures.

B. Eleventh instar larvae

Allatectomy of eleventh instar larvae resulted in adults which had normal-appearing antennae with sexual dimorphism (table 2B, fig. 5). Sexual dimorphism also developed in the contact chemoreceptor populations of adults from allatectomized eleventh instar larvae and eleventh instar larvae derived from sham-operated tenth instar larvae (table 2A). These results reinforce the notion that experimental intervention released a latent sexual dimorphism of contact chemoreceptors in this strain of *P. americana*.

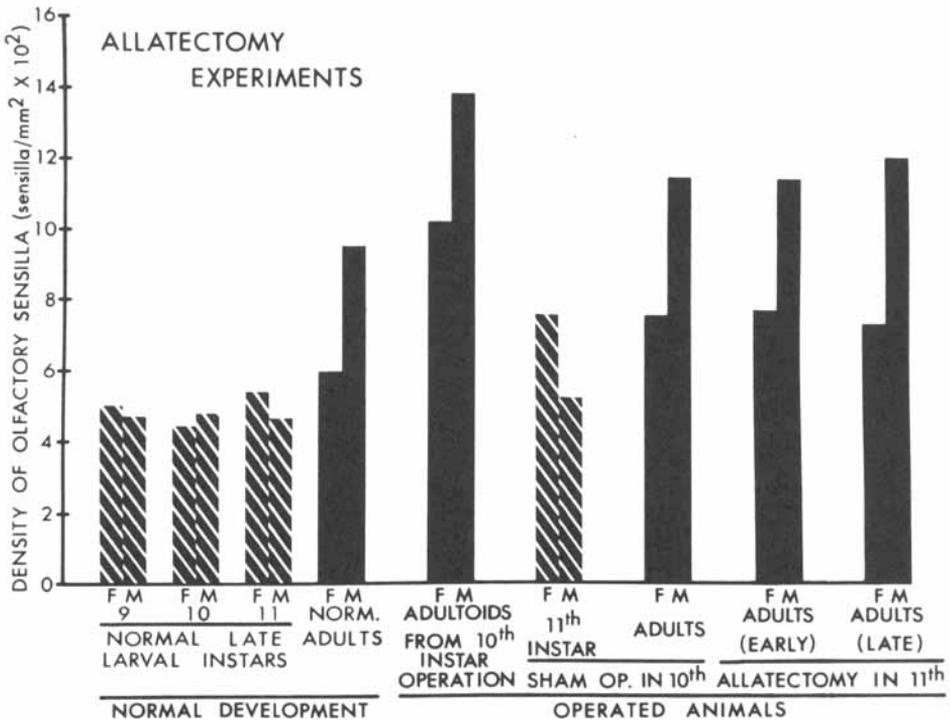


Fig. 5 Results of the allatectomy experiments with respect to the density of olfactory sensilla. Adultoids derived from allatectomized tenth instar larvae developed sexual dimorphism and also showed a stimulation of sensillar differentiation in both males and females. Allatectomized eleventh instar larvae also developed into adults with antennal sexual dimorphism, whether the operation was done early or late in the instar. Sham-operated tenth instar larvae ecdysed to eleventh instar larvae which subsequently ecdysed to adults. Sexual dimorphism was present in the sham-operated adults, but not in sham-operated eleventh instar larvae. The larger average density in female eleventh instar larvae stems from the inclusion in the average of one female antenna with an exceedingly large population of olfactory receptors.

Juvenile hormone experiments

A. Whole-animal exposure to exogenous JH-M (table 3, figs. 3A, 6)

Sixty-eight percent of the eleventh instar larvae exposed to exogenous JH-M ecdysed to supernumerary, (twelfth instar) larvae. The remaining 32% ecdysed to larvoids characterized by abnormally large wing pads. Twelfth instar larvae showed no sexual dimorphism of either contact chemoreceptors or olfactory sensilla, but, surprisingly, the larvoids developed sexual dimorphism in both characters.

Adults derived from twelfth instar larvae which had been removed from the influence of JH-M, developed sexual dimorphism of the olfactory sensilla. Semi-adults derived from twelfth instar larvae also de-

veloped sexual dimorphism of olfactory sensilla despite being under the continuing influence of JH-M. Semi-adults were often active and healthy-looking during the first few weeks after ecdysis, but tended to die sooner than normal insects kept under the same conditions. In each case cited above (larvoids, semi-adults, and adults derived from twelfth instar larvae) the existence of sexual dimorphism was statistically significant at the 1% level (Student's t-test, two tailed).

B. Bilateral JH-M treatment of male antennae in eleventh instar

Topical treatment of both antennae with exogenous JH-M at concentrations of 1:100 and 1:20 resulted in a decrease in the total

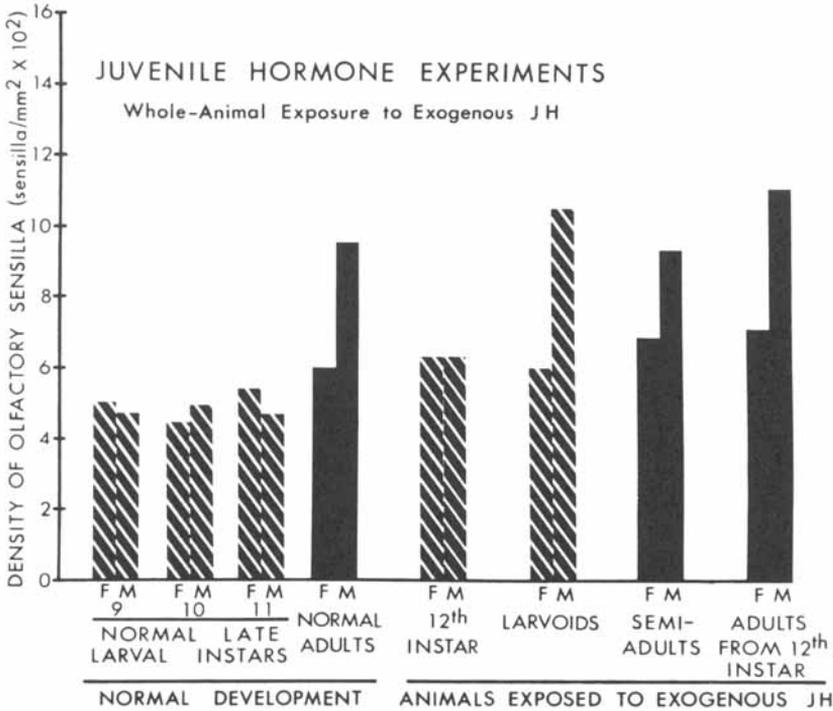


Fig. 6 Results of whole-animal exposure to juvenile hormone-mimic with respect to the density of olfactory sensilla. Twelfth instar larvae and larvoids were produced by exposure of eleventh instar larvae to JH-M. The twelfth instar larvae lacked sexual dimorphism, but the larvoids (which also had exceptionally large wing pads) did develop sexual dimorphism. Semi-adults produced by continued exposure of twelfth instar larvae to JH-M displayed sexual dimorphism, as did adults from twelfth instar larvae which were removed from the influence of JH-M.

TABLE 3

Effects of juvenile hormone-mimic (JH-M) on antennal development in *Periplaneta americana*. Whole-animal exposure to exogenous JH-M in culture container (using strain P.a./MR). All figures are means

Stage	Sex	N	Antennal surface area (mm²)	Total number of contact chemoreceptors	Density of contact chemoreceptors (/mm²)	Total number of olfactory sensilla	Density of olfactory sensilla (/mm²)
Twelfth instar (from exposed eleventh instar)	M	3	35.3	8,090	229	22,190	628
	F	3	31.5	6,790	216	19,830	630
Larvoid (from exposed eleventh instar)	M	3	30.1	13,050	434	31,440	1,045
	F	4	39.2	8,140	207	23,460	598
Semi-adult (from exposed twelfth instar)	M	5	33.3	10,330	310	30,930	929
	F	3	39.1	9,170	235	26,690	683
Adult (from twelfth instar removed from JH influence)	M	3	32.5	8,930	274	35,775	1,100
	F	2	26.7	7,880	295	18,740	703

number and density of olfactory sensilla at the adult stage, accompanied by some suppression in the number of contact chemoreceptors (table 4B). Untreated antennae from the same strain and culture as the ex-

perimental insects were counted for comparison (table 4A). The difference in the number of olfactory sensilla between control antennae and antennae treated with concentrations of 1:100 and 1:20 was

TABLE 4

Effects of juvenile hormone-mimic (JH-M) on antennal development in Periplaneta americana. Topical application to antenna. All figures are means

	Sex	N	JH-M conc.	Total number of contact chemo-receptors	Density of contact chemoreceptors (/mm ²)	Total number of olfactory sensilla	Density of olfactory sensilla (/mm ²)
A. Normal antennae (strain P.a./UR)							
	M	10	Untreated	12,750	407	38,470	1,228
B. Effects on adult antennae after bilateral JH-M treatment of the antenna in the eleventh larval instar (using strain P.a./UR)							
	M	18	1:20	11,070	356	30,680	986
	M	12	1:100	11,210	359	31,820	1,019
	M	4	1:1000	10,110	379	28,820	1,080
	F	10	1:20	8,870	336	18,690	708
C. Effects on adult male antennae after unilateral JH-M treatment of the antenna in the eleventh larval instar (using strain P.a./UR)							
Treated	M	11	1:20	12,370	386	30,510	952
Untreated	M	11	1:20	12,230	390	39,070	1,246
Treated	M	3	1:100	11,510	330	33,870	972
Untreated	M	3	1:100	10,980	362	35,300	1,164
Treated	M	7	1:1000	11,340	408	32,380	1,167
Untreated	M	7	1:1000	11,340	380	34,130	1,143
Treated	F	3	1:20	8,340	344	18,400	760
Untreated	F	3	1:20	8,640	374	17,520	758

statistically significant at the 10% level (t-test). The 1:1000 concentration also appeared to suppress the number of olfactory sensilla (table 4B), but this was not statistically supportable. All other morphological features of the treated insects appeared entirely normal and fully adult. Female antennae treated with 1:20 JH-M seemed unaffected compared with female P.a./UR antennae examined in an earlier study (table 4B; Schafer and Sanchez, '76).

Topical JH-M treatment *did not* completely inhibit the differentiation of new olfactory sensilla at the adult stage (fig. 7). A large number of new olfactory sensilla appeared even after treatment with the highest JH-M concentration. However, since increases normally occur at larval ecdyses, it may be unreasonable to expect any level of JH-M treatment to totally inhibit the differentiation of olfactory sensilla. The total number of olfactory sensilla normally increases an average of 1.27 times at each larval ecdysis and 2.29 times at the adult ecdysis (Schafer and Sanchez, '76). With the 1:20 JH-M treatment, olfactory

sensilla increased approximately 1.7 times (using data from both Schafer and Sanchez, '76 and the present experiments).

C. Unilateral JH-M treatment of male antennae in eleventh instar

Topical unilateral treatment of male antennae in the eleventh instar with JH-M concentrations of 1:100 and 1:20 decreased the total number and density of olfactory sensilla when compared to contralateral antennae treated with only ethanolic solvent (table 4C, fig. 7). All other morphological features were unaffected by the treatment. The difference between untreated and treated antennae was statistically significant at the 2% level for the 1:20 JH-M treatment and at the 10% level for the 1:100 JH-M treatment (t-test). The 1:1000 treatment had no effect. Female antennae were also unaffected by a 1:20 JH-M treatment.

D. Effects of topical JH-M on the meristal segments' sensilla

Meristal segments, which are the most

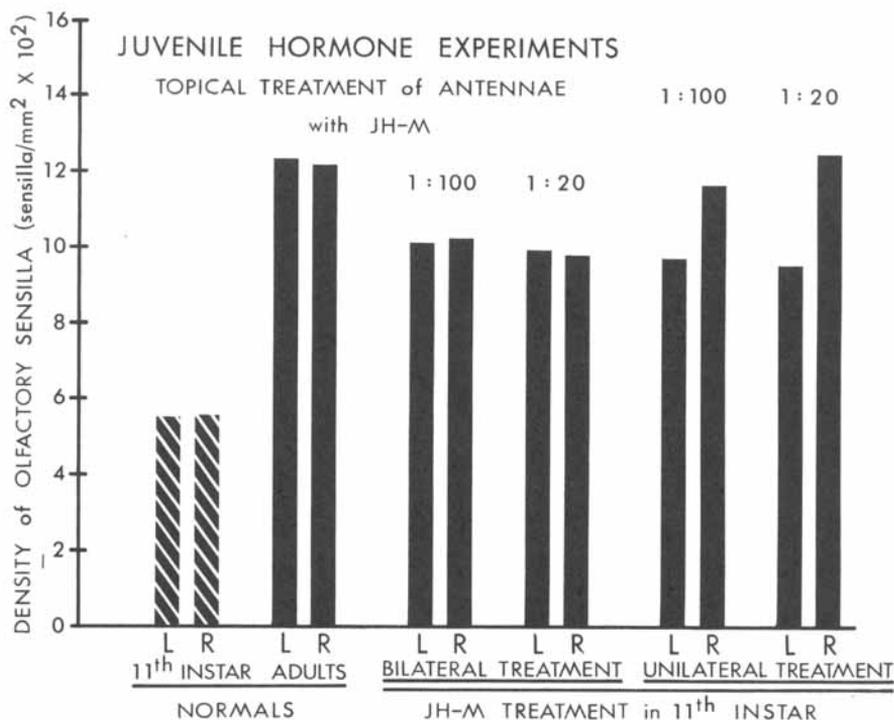


Fig. 7 Results of topical treatment of the antennae of eleventh instar larvae with juvenile hormone-mimic. All antennae in this figure are from males. The average difference between the left and right antennae (L and R) of normal eleventh instar males and adult males is shown at left. Shown at right is the density of olfactory sensilla on adult male antennae after treatment of the antennae in the eleventh instar with JH-M at two concentrations, 1:100 and 1:20. In one group, both antennae were treated with JH-M (bilateral treatment). In a second group, the left antenna was treated with JH-M in ethanol solution while the right was simultaneously treated with the ethanol solution alone (unilateral treatment). Three different concentrations (1:1000, 1:100, and 1:20) were used, but only concentrations of 1:100 and 1:20 were effective, and only they are shown in the figure. Juvenile hormone-mimic treatment inhibited the differentiation of olfactory sensilla whether applied bi- or unilaterally.

proximal segments of the antennal flagellum, normally lack olfactory sensilla during the larval period, but acquire a large number at the adult stage. *P. americana* displays sexual dimorphism in this respect, i.e., adult males have olfactory sensilla on all meristal segments, adult females do not (Schafer and Sanchez, '76). Most male antennae treated with JH-M in the terminal larval instar showed substantially fewer meristal olfactory sensilla than normal males. In antennae from bilaterally-treated animals (concentration 1:20), the average inhibition of the number of olfactory sensilla on segments 4 through 14 was 73% of the normal value. On unilaterally-

treated animals (concentration 1:20), segments 4 through 14 of the JH-M treated antennae had 70% of the normal number of olfactory sensilla. The untreated antennae of unilaterally-treated animals had 104% of the normal population of olfactory sensilla, indicating that there was no inhibition of differentiation by the control treatment (ethanol-dipping). No instance of total inhibition of olfactory sensillar development was observed on either the bilaterally-treated or the unilaterally-treated antennae.

Effects of ecdysterone and ethanol

Ecdysterone treatment had no effect on

antennal development under the conditions of these experiments. Ethanol treatment also had no effect.

DISCUSSION

These experiments employed three methods of altering juvenile hormone titers in larval cockroaches: (1) maintenance of JH secretion induced by the trauma of unilateral antennectomy; (2) surgical removal of the corpora allata, the source of JH; and (3) application of exogenous JH-M. The first method (antennectomy) produced results which are consistent with — but do not directly support — the idea that JH suppresses the development of sexual dimorphism in *P. americana* antennae. Juvenile hormone is implicated because the major hormonal event influencing the emergence of the adult pattern is derepression of adult characteristics as the corpora allata cease or profoundly reduce production of JH during the terminal instar (Wigglesworth, '64; cf. Slama, '75).

The second method, i.e., allatectomy of tenth instar larvae, produced adultoids with adult-like antennae. This result supports the hypothesis that JH controls sensillar differentiation. The number of olfactory sensilla on male antennae more than doubled in this case, as might be expected, since near-doubling also occurs at the normal metamorphosis. The number of olfactory sensilla on allatectomized female antennae also increased substantially, nearly attaining the level of normal female adults despite a drop in the antennal surface area. We assume that the male genome predisposes its larger response to the removal of JH. The results of sham operations on tenth instar larvae and allatectomy of eleventh instar larvae indicate that surgical trauma had little influence on antennal sensillar development.

The results of whole-animal exposure to JH-M suggest that a high titer of JH is necessary to inhibit the development of the male pattern. Larvoids, outwardly distinguishable only by their enlarged wing pads, developed antennal sexual dimor-

phism. The production of chimerical larvoids and semi-adults with mixtures of larval and adult features corresponds to the patterns of sensitivity to JH observed in other insects (e.g., Gilbert and Schneiderman, '60; Postlethwait, '74). The wings of *Rhodnius* are least susceptible to the neotenic effects of JH among various morphological characters controlled by JH (Wigglesworth, '64). These experiments indicate that the antennal system of *P. americana* has a higher threshold than the wings.

The separation of insects treated with JH-M into classes (larvoids, semi-adults, and adults) was clearly appropriate in *Leucophaea maderae* (Shafer and Sanchez, '74), but was more arbitrary in *P. americana*. The categories were retained, however, to facilitate discussion. The range of sensitivity of JH-M among different organs was obvious in both species by examining the intermediate forms (larvoids and semi-adults). No cuticular mosaics were detected in either *Leucophaea* or *Periplaneta*, including the experiments on topical application of JH-M to the antennae. Instead, variation took the form of trends: smaller or larger wings of uniform appearance, or fewer or more antennal sensilla without abnormal regional variations in density along the antennae. We conclude that once the threshold for sensillar differentiation is attained, the sensilla appear — morphologically complete and without abnormalities.

The most compelling evidence for the inhibitory role of JH is provided by the experiments employing topical application of JH-M to the antennae. However, these experiments are marred to some extent by the variability of responses of different insects to the same concentration of hormone. A majority of antennae were inhibited by JH-M treatment, but a few were little affected.

Variability in experiments with topically-applied JH-M can be plausibly explained by the concept of the critical period. Each tissue or organ is supposed to pass through a stage in its developmental program during which it is sensitive to the influence of

JH, but after which it becomes insensitive (Willis, '74). This could easily explain the variability observed, because eleventh instar larvae of varying ages were used. Insects of differing known ages could be treated to determine the critical period, but such a study would require the laborious examination of a large number of antennae.

One fact deserves special attention: Topically-applied JH-M inhibited the differentiation of olfactory sensilla on the meristal segments of adult males, but never completely. This finding is important because the olfactory sensilla which appear on the meristal segments of male antennae at the adult stage are the only sensilla which can be absolutely identified as having differentiated at the terminal ecdysis. Therefore, we know with certainty that none of the experiments with topically-applied JH-M totally succeeded in blocking the development of new olfactory sensilla at the adult stage, although inhibition was observed in most experiments.

Again it seems plausible to invoke the concept of critical period to explain the less than complete JH-M inhibition of the differentiation of meristal sensilla. A number of other factors, such as variation in antennal permeability or differences in the cuticular waxes which might be abraded in handling or dissolved by the ethanol, might also have contributed. In addition, a unique difficulty is encountered in treating antennae with JH-M which is not shared by experiments involving topical application to other body parts. Cockroaches continually groom their antennae by running them through their mouth parts. This behavior increases after handling or experimental procedures and would transfer some of the JH-M from the antennae to the mouth. For this reason, antennae were rinsed after topical JH-M treatment. The rinse and/or subsequent cleaning by the animal itself may also help to explain the incomplete inhibition by topical JH-M treatment.

The hypothesis supported by these experiments may be stated as follows: Juvenile hormone regulates the development

of sexual dimorphism in *P. americana* by inhibiting the differentiation of the full male complement of olfactory sensilla during larval development. The increase in olfactory sensilla in adult males could be aided by lower titers of JH during male adult development compared with the JH levels in developing females. Assuming this hypothesis is correct, two corollaries follow: (1) the sensilla which are inhibited by JH are likely to contain sex attractant receptors; and (2) the inhibition of attractant receptor differentiation during larval development results in the inability of larvae to respond behaviorally to the sex attractant. The data presented here argue for the correctness of the hypothesis, while the papers which follow (Schafer, '77a,b) explore the corollaries concerning receptor specificity.

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