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

I. SEXUAL DIMORPHISM IN THE DISTRIBUTION OF ANTENNAL SENSE ORGANS IN FIVE SPECIES

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ABSTRACT   Sexual dimorphism in the distribution of antennal sense organs is common among adults of the genus *Periplaneta*. In three out of the four strains of *Periplaneta americana* examined, adult males had more contact chemoreceptors than females. In the fourth strain of *P. americana* and in *P. australasiae*, *P. brunnea*, *P. fuliginosa*, and *P. japonica*, no statistically supportable sexual dimorphism of contact chemoreceptors was found. However, in all strains and species of *Periplaneta* examined, sexual dimorphism was found in the total number and/or density of olfactory sensilla. Male adults had nearly twice as many olfactory sensilla as female adults. These observations are consistent with the behavioral observation that males within the genus *Periplaneta* rely on the reception of an airborne pheromone for the initiation of courtship behavior. In *P. americana*, where sexual dimorphism was found in the contact chemoreceptors, contact stimuli release the full wing raising display and presentation in males during courtship.

Cockroaches of the genus *Periplaneta* employ sex attractants to initiate the courtship behavior which leads to mating (Roth and Willis, '52a; Barth, '61, '70; Roth and Barth, '67; Frazier, '70; Simon, '71). The initial step in courtship behavior is reception of a female-produced sex attractant by the male, the antennae of which are sensitive to the attractant (Roth and Willis, '52a). This initial event leads to excitement in the male and oriented locomotion in search of the pheromone source. Location of the female by the male is followed by a stereotyped sequence of male-female interactions which may or may not lead to successful mating (Barth, '70; Simon, '71). A long series of unsuccessful attempts has been made to isolate and identify the chemistry of the sex attractant of the American cockroach, *Periplaneta americana* (Jacobson, '72). Whatever the reasons for the failure of this line of research so far, it now seems likely that at least two (possibly synergistic?) compounds are involved in *P. americana* sex attraction. The reader should bear this possibility in mind in subsequent discussions which refer to *the* sex attractant.

The antennae of *Periplaneta* mediate a variety of functions (Schafer and Sanchez, '73; Lambin, '73; Sass, '73, '74; Boeckh, '74; Boeckh et al., '75), but sex attractant reception is of paramount importance to male sexual behavior (Roth and Willis, '52a). In line with the reliance of the male on olfactory cues, sexual dimorphism has been reported in the antennae of adult *Periplaneta americana*, but not in larvae (Schafer and Sanchez, '73; Lambin, '73). The antennae of adult males have nearly twice as many olfactory sense organs as adult females (3.9×10^4 olfactory sensilla in males versus 2.2×10^4 sensilla in females). Both male and female larvae of the terminal instar have about 1.7×10^4 olfactory sensilla, which indicates that morphologically observable sexual dimorphism develops only at the final ecdysis leading to the adult stage (Schafer and Sanchez, '73). Morphological descriptions of the anten-

nal sensilla of *P. americana* have appeared (Schafer and Sanchez, '73; Lambin, '73), as well as several studies on the antennal sensilla of other species of cockroaches: *Blattella germanica* (Eggers, '24, 28; Roth and Willis, '52b; Ishii, '71; Campbell, '72), *Gromphadorhina protentosa* (Slifer, '68), *Gromphadorhina brunneri* (Hintze-Podufal and Otto, '75), *Leucophaea maderae* (Schafer, '71), and *Arenivaga sp.* (Hawke and Farley, '71a,b). In general, these studies have disclosed two major classes of chemoreceptors on cockroach antennae: thin-walled hairs with an olfactory function and thick-walled hairs which probably function in contact chemoreception and tactile reception. An exception to this general pattern is found in the antennae of the desert burrowing cockroach, *Arenivaga*. Probable olfactory sensilla in *Arenivaga* have much thicker walls than are found in the olfactory sensilla of other insects (Hawke and Farley, '71a,b). A wide variety of mechanoreceptive sensilla is also present on cockroach antennae, as well as receptors for hygroreception and thermoreception (Gunn and Cosway, '38; Roth and Willis, '52b; Loftus, '66, '68, '69). A unique type of wavy tactile hair is present on adult male antennae of *Gromphadorhina portentosa* (Slifer, '68) and *Gromphadorhina brunneri* (Hintze-Podufal and Otto, '75).

The postembryonic development of the antennae of *Periplaneta americana* was misinterpreted by Bugnion ('17, '21), but has been correctly described by Campbell ('28), Haas ('55), and Schafer and Sanchez ('73). Normal antennal development has also been described in several other species, including *Blabera craniifer* (Urvoy, '63), *Blattella germanica* (Campbell and Priestley, '70), and *Leucophaea maderae* (Schafer, '73). These studies indicate that normal growth takes place by the addition of new segments at each ecdysis. The meriston (or third segment) produces from 5 to 30 new segments at each ecdysis. These segments (meristal segments) then undergo binary division at the following ecdysis.

The differentiation and distribution of olfactory receptors on cockroach antennae is influenced by the juvenile hormone (Schafer and Sanchez, '74). The hormone also influences antennal regeneration and the formation of pattern in the antennal sensilla (Schafer, '73). Antennal regeneration was mentioned by several early workers (Heinecken, 1829; Brindley, 1898; Illingworth, '17), and has been most intensively studied in *Periplaneta americana* (Haas, '55), *Blabera craniifer* (Urvoy, '63), and *Leucophaea maderae* (Schafer, '73). These studies indicate that the antennae of larval cockroaches have a powerful regenerative potential. New segments may be produced during regeneration by the meristal segments, the meriston, the scape and pedicel, and the antennifer or head capsule. Antennectomy prolongs larval development, both by lengthening the larval instar during which the operation is performed and by inducing one or more supernumerary larval instars if the operation is performed early in the terminal larval instar (Schafer, '73).

The present paper describes the development of sexual dimorphism in the antennae of *Periplaneta americana* and its occurrence in the antennae of four other species within the genus *Periplaneta*. Subsequent papers will consider the influence of juvenile hormone on the antennal sensory system, the sexual behavior of the five species, and the electrophysiology of the antennal receptors.

MATERIALS, METHODS AND NOMENCLATURE

The cockroaches used in this study were obtained from the following sources: *Periplaneta americana* (L.) — *P.a.*/MR from P. W. Winston at the University of Colorado, Boulder, and possibly derived from the cultures which were until recently kept by L. M. Roth at the U. S. Army Natick Laboratories — *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 — *P.a.*/WE, a strain with white eyes,

from F. W. Fisk at The Ohio State University, Columbus, and probably derived from white-eyed specimens obtained from a coal mine in South Wales (Jefferson, '58); *Periplaneta japonica* from D. Alsop at Queens College of the City University of New York, Flushing; and *Periplaneta australasiae* (Fabricius), *Periplaneta brunnea* Burmeister, and *Periplaneta fuliginosa* (Serville) from F. W. Fisk. We thank Drs. Adler, Alsop, Bodenstein, Fisk, Sternburg, and Winston for the specimens they donated.

The development data on *P.a.*/MR were obtained by segregating oöthecae in separate culture jars, and removing a few newly-emerged insects for study at each stage of postembryonic development. Data from a previous study on *P.a.*/MR (Schafer and Sanchez, '73) were combined with new data to give a more precise picture of antennal development in this strain of *P. americana.* Specimens of the other strains of *P. americana* and the other species were obtained from mass cultures.

Some antennae were fixed within 30 minutes of ecdysis, and they produced the best whole mounts. Newly-ecdysed antennae were fixed for six hours in a modified Carnoy-Lebrun fixative (25 ml ethanol + 15 ml glacial acetic acid + 15 ml chloroform + 4.0 g mercuric chloride), washed in 50-50 ethanol-isopropyl alcohol, dehydrated in ethanolic series, cleared in xylol/phenol (3/1 + 1% ethanol), and mounted in Hoyer's solution (50 g water + 30 g gum acacia + 200 g chloral hydrate + 20 g glycerol) for further clearing and examination. Tanned (darkened) antennae were first bleached in 3% hydrogen peroxide for 24-48 hours at 60°C until cleared, then treated according to the method above. The nearly transparent whole mounts were used to determine the total number of antennal sensilla in antennae of each stage and the surface area of the antenna. The total number of each type of sensillum was extrapolated from absolute counts of all the sensilla on 20-30% of the segments in each antenna.

Permeable areas of antennal sensilla

were examined by using several different methods. Slifer's ('60) crystal violet technique was applied to newly-ecdysed antennae fixed in 10% formalin. Porous areas were also identified by the presence of reduced silver deposited in the following way. Freshly-excised, newly-ecdysed antennae were washed three times in 10% acetone and dipped in 0.1 M $AgNO_3$ for two minutes. After washing three times in distilled water, the antennae were immersed in Kodak Microdal-X developer for about five minutes. When sufficient darkening was observed under a powerful dissecting microscope, the antennae were rinsed in 3% acetic acid for one minute, then dehydrated, cleared, and mounted as previously described. A modification of the cobalt staining method of Pitman et al. ('72) was also used. Freshly-ecdysed antennae were mounted for ease in handling by fixing their bases to toothpicks with melted paraffin. The antennal flagellum was dipped three times in 10% acetone and placed in fresh 1 M $CoCl_2$ for three hours. After staining, the antennae were washed in running distilled water for one minute or less and placed in 1% ammonium sulfide for one hour. The antennae were then washed in running distilled water for ten seconds and dehydrated and mounted for observation as previously described.

The extrusion of antennal fluids through cuticular pores in the sensilla was also examined (cf. Slifer et al., '57). Freshly-excised antennae (either tanned or newly-ecdysed) were sealed at the cut base with hot paraffin and placed in mineral oil between a microslide and cover glass. The antennal sensilla were observed under the microscope while the cover glass was gently depressed. The internal pressure created by deforming antenna forced fluids out through pores in some of the chemoreceptive sensilla.

Scanning electron microscopy (SEM) was also employed in an attempt to detect porous areas of the antennal sensilla. Preparation for SEM was minimal. The antennae were excised, dried overnight over anhydrous calcium chloride, mounted on

stubs with siver-bearing paint (Silver Print, GC Electronics No. 21-2, Walsco No. 36-1), gold coated, and examined with the scanning electron microscope (JEOL JSM-2). Great care was taken to avoid antennal contact with *any* surface during preparation, as such contacts always resulted in the electrostatic accumulation of dust particles.

The nomenclature used in this paper and succeeding papers is as follows:

1. *Segment* is used to denote any subdivision of the antenna including the scape, pedicel, meriston and the many divisions of the antennal flagellum. The term *subsegment*, classically applied to the divisions of the antennal flagellum of insects (Snodgrass, '35), is omitted here to simplify description and discussion.

2. The *meriston* (after Campbell and Priestley, '70) is the third antennal segment and is the principal growth center in larval antennae.

3. The *meristal segments* are the new segments which emerge from the meriston at each ecdysis and undergo binary division at the following ecdysis. This term is synonymous with the "primary zone"

(Primärzone) of Haas ('55) and the "meristal annuli" of Campbell and Priestley ('70). The meristal segments are easily recognized at the base of the antennal flagellum because they are noticeably shorter than more distal segments and carry fewer sensilla.

4. The *antennal flagellum* consists of all antennal segments distal to the meriston, including the meristal segments.

5. The *terminal ecdysis* is defined as the ecdysis from which the adult emerges. A *larval ecdysis* is an ecdysis from which an immature nymph or larva emerges.

RESULTS AND DISCUSSION

Contact chemoreceptors

Thick-walled chemoreceptive sensilla, previously designated sensilla chaetica B (Schafer and Sanchez, '73), occur on all segments of the antenna. The following are essential features of such sensilla: a hollow, thick-walled and fluted shaft of variable length (35-250 µ) containing an inner cuticular sheath in its proximal half; a flexible socket at the base, which allows considerable movement back and forth in a longitudinal plane, but more limited move-
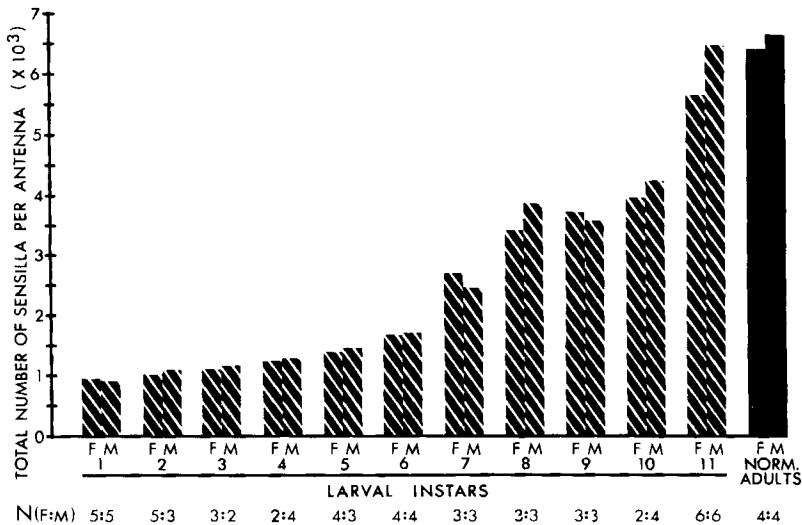

Fig. 1 Normal development of the total number of contact chemoreceptors on the antennae of *Periplaneta americana*. Crosshatched bars indicate larval stages and solid bars indicate adults. Individual counts of male and female antennae are indicated by F and M. The number of male and female antennae counted is given below as N(F:M).

Fig. 2   Normal development of the density of contact chemoreceptors on the antennae of *Periplaneta americana*. Notation as in figure 1.

Fig. 3   Total number of contact chemoreceptors on the antennae of four strains of *Periplaneta americana* and four other species within the genus *Periplaneta*. The crosshatched bars indicate the population of contact chemoreceptors in the eleventh instar of *P. americana*. Other notations as in figure 1.

traordinarily large in comparison with the other strains (figs. 3-4), and is accompanied by a higher density of olfactory sensilla (fig. 8). In behavioral tests of males responding to female-produced sex attractant, *P.a.*/UR males produced higher responses levels than males of the three other strains (Schafer, unpublished data). Whether or not the greater number of contact chemoreceptors and olfactory receptors contributed to the behavioral difference is unknown, and could just as well be at-

tributed to the greater degree of vigor and general activity which the *P.a.*/UR males also displayed. The density of contact chemoreceptors on antennae of the caver- nicolous white-eyed strain, *P.a.*/WE, is not surprising in view of the fact that these sensilla also serve as tactile receptors (Schafer and Sanchez, '73).
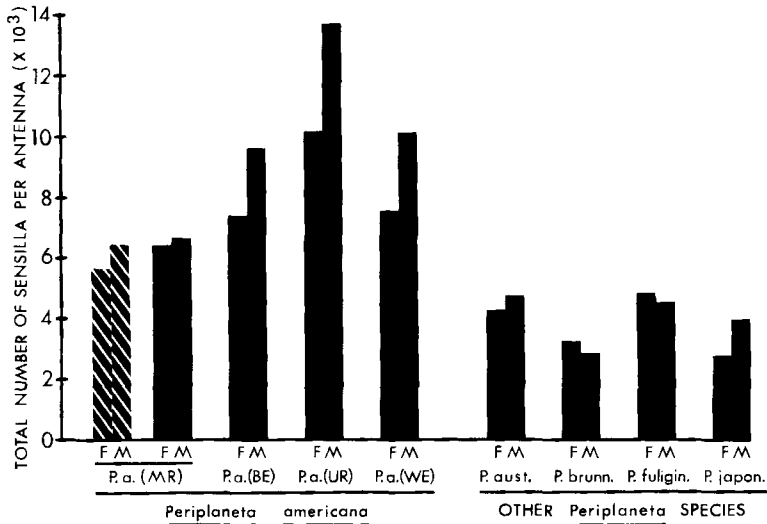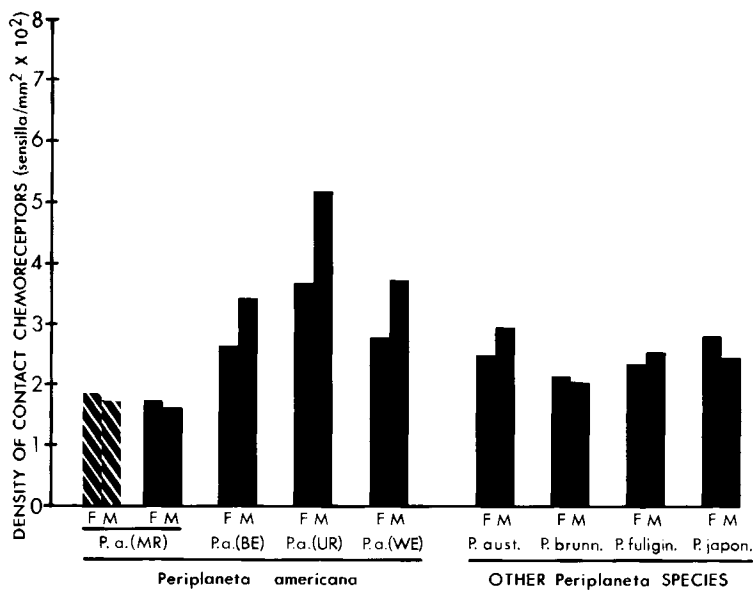

Fig. 4 Density of contact chemoreceptors on the antennae of four strains of *Periplaneta americana* and four other species within the genus *Periplaneta*. Notations as in figure 3.

TABLE 2

Sexual dimorphism in antennae of the genus Periplaneta

Stage	Sex	Antennal surface area (mm²)	Standard deviation	Total contact chemoreceptor sensilla	Standard deviation	Total olfactory sensilla	Standard deviation	N
P. a./MR	M	37.8	2.7	6,480	1,480	17,490	860	6
Instar 11	F	30.6	12.0	5,660	1,460	16,440	990	6
P. a./MR	M	41.0	5.9	6,640	640	38,910	240	4
Adult	F	37.1	2.2	6,410	2,100	22,070	110	4
P. a./BE	M	28.1	0.3	9,650	270	33,420	430	3
	F	28.1	1.5	7,420	990	16,730	1,620	3
P. a./UR	M	26.5	1.6	13,720	240	40,820	2,100	3
	F	27.7	0.9	10,200	1,120	22,650	2,690	3
P. a./WE	M	27.2	1.9	10,170	280	25,440	1,890	3
	F	27.4	0.1	7,590	130	18,100	1,260	3
P. aust.	M	16.2	1.2	4,770	700	25,200	3,100	3
	F	17.2	2.6	4,290	190	16,770	510	3
P. brunn.	M	13.9	0.2	2,830	270	19,130	1,870	3
	F	15.3	0.7	3,260	160	11,780	340	3
P. fulig.	M	17.7	2.6	4,530	470	22,800	3,510	3
	F	20.8	3.8	4,860	430	16,710	2,220	3
P. japon.	M	16.1	4.2	3,960	410	18,780	950	3
	F	9.9	1.2	2,780	350	10,950	750	3

No statistically supportable sexual dimorphism is apparent in the total number or density of contact chemoreceptors on the antennae of three other species of Periplaneta examined: *P. australasiae, P. brunnea*, and *P. fuliginosa* (figs. 3-4). Antennae of male *Periplaneta japonica* are somewhat larger than those of females (table 2), and this difference accounts for the larger total number of contact chemoreceptors on male antennae in this species (fig. 3).

Olfactory sensilla

Olfactory sensilla (sensilla trichodea and sensilla basiconica of Schafer and Sanchez, '73) occur in large numbers only on the antennal flagellum. The following are essential features of *Periplaneta* olfactory sensilla: a hollow, thin-walled shaft or peg 5-60 μ in length on a fixed base; a tip which may be sharp (trichoid), or sometimes blunt (basiconic); with cuticle porous to crystal violet making up the distal two-thirds of the hair; and typically innervated by two neurons (fig. 9).

The olfactory sensilla share a similar morphology and distribution on the antennae of all five species examined. In larval antennae they are most numerous on the distal part of the flagellum, and do not occur in any substantial numbers on the meristal segments. Olfactory sensilla do not occur at all on the scape and pedicel at any stage of development, but may occur infrequently on the meriston of adult males.

The distribution of olfactory sensilla changes radically at the terminal ecdysis. Adult males of all species examined have olfactory sensilla present in large numbers (50 or more per segment) on all segments distal to the meriston. In contrast, female adults do not have olfactory sensilla on the base of the antenna until about the fifth segment distal to the meriston, and then only in reduced numbers in comparison with the male (about 10 olfactory sensilla per segment).

In the most-studied strain of *Periplaneta americana*, *P.a./MR*, sexual dimorphism does not develop until the adult stage (figs. 5-6). During larval development, the antennal surface area increases by an average of 1.38 times at each ecdysis through the eleventh instar while olfactory sensilla in-
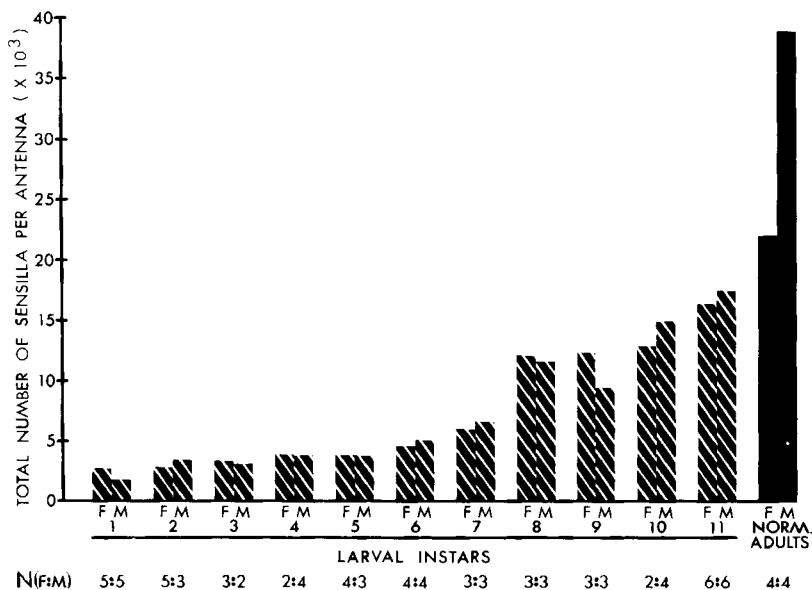

Fig. 5 Normal development of the total number of olfactory sensilla on the antennae of *Periplaneta americana*. Notations as in figure 1.
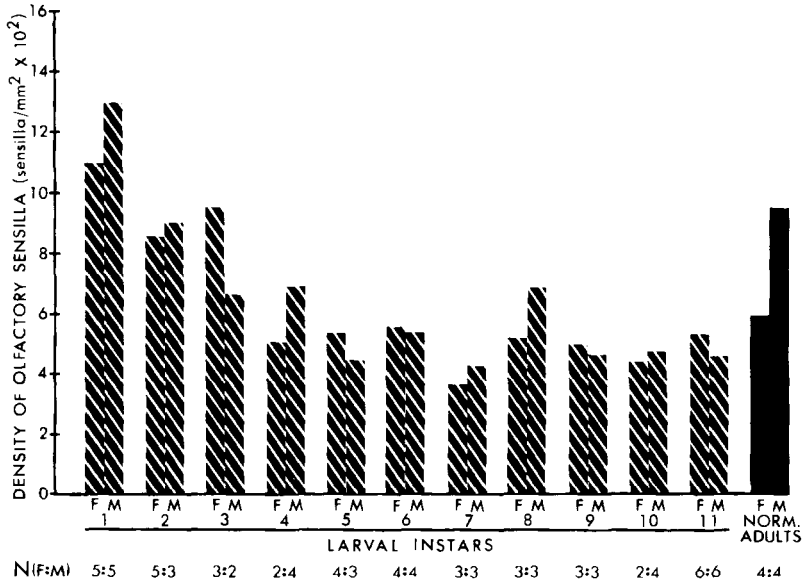
Fig. 6 Normal development of the density of olfactory sensilla on the antennae of *Periplaneta americana*. Notations as in figure 1.

crease in total number by an average of 1.27 times at each ecdysis (table 1). This rate of increase in olfactory sensilla is maintained through adulthood in female antennae, increasing by 1.30 times at the terminal ecdysis. In the male adult, however, the increase is 2.29 times (table 1, fig. 5). *Thus, a direct relationship exists between the increase in antennal surface area and olfactory sensilla, except at the terminal ecdysis in the male.*

The relationship between surface area and the development of olfactory sensilla is further evident when the density of olfactory sensilla is followed through postembryonic development (fig. 6). Since the increase in antennal surface area at each ecdysis is slightly greater than the increase in the total number of olfactory sensilla (table 1), the density of olfactory sensilla falls during early larval development until it reaches a constant level of about 4-600 sensilla/mm² in the later instars (fig. 6). At the adult ecdysis, the female antenna stays at this level, but the male nearly doubles the density of olfactory sensilla, reaching nearly 1,000 sensilla/mm².

Sexual dimorphism in the total number

of antennal olfactory organs is evident in all of the four strains of *Periplaneta americana* examined (fig. 7). The density of olfactory sensilla and the difference between males and females is greatest in *P.a./UR* and smallest in the white-eyed strain, *P.a./WE* (fig. 8). A comparison of the sensillar populations of the four strains (figs. 7-8) indicates that considerable variation occurs, but all have a substantial sexual dimorphism.

Male and female *Periplaneta americana* possess more antennal olfactory sensilla than their counterparts in the other four species examined (fig. 7). This is attributable to the smaller size of the antennae of the other species when compared to *P. americana* (table 2). The density of antennal olfactory organs is well within the range of variation demonstrated in the several strains of *Periplaneta americana* (fig. 8). *Periplaneta japonica* is of note because its sexual dimorphism takes a different pattern than the other species. In *P. japonica* the difference in sensillar populations between male and female antennae stems from a difference in the gross size of the antennae of males and females, not a
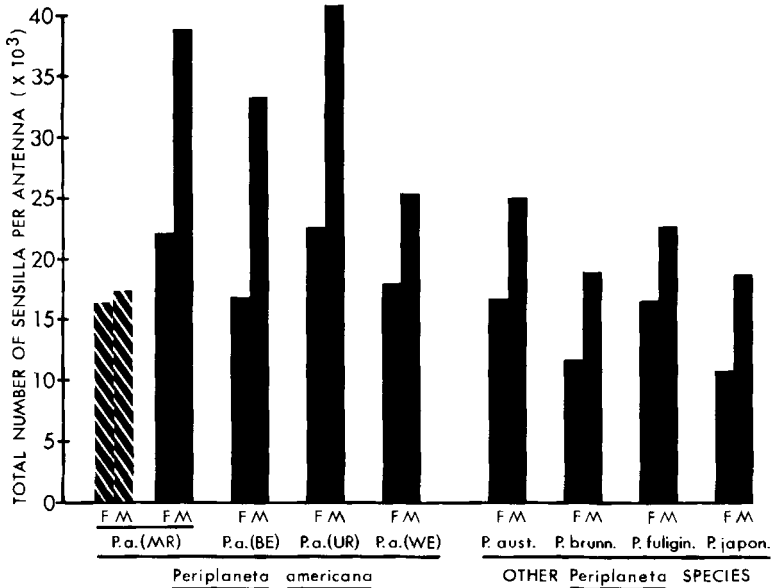
Fig. 7   Total number of olfactory sensilla on the antennae of four strains of *Periplaneta americana* and four other species within the genus *Periplaneta*. Notations as in figure 3.

difference in the density of sensilla (table 2, fig. 8).

The meaning of variability in sensillar distribution

Considerable variation exists in the density of olfactory sensilla among the five species examined, and among the different strains of *Periplaneta americana*. Such variability would be expected to emerge in quantitative measurements of any morphological character. However, the variability in the distribution of *contact chemoreceptors* is qualitatively different from the variability of olfactory sensilla. The initial study of *P.a.*/MR indicated no sexual dimorphism in contact chemoreceptors (figs. 1,2; Schafer and Sanchez, '73). However, a difference appeared in the data from *P.a.*/BE, and an obvious sexual dimorphism was apparent in the two other strains, *P.a.*/UR and *P.a.*/WE (figs. 3, 4). Application of Student's t-test (two-tailed) to the male-female difference in the total number of contact chemoreceptors gave values of 3.77 for *P.a.*/BE, 7.31 for *P.a.*/UR, and 12.59 for *P.a.*/WE. These results indicate that the

measured differences are statistically significant at the following levels: 10% for *P.a.*/BE, 2% for *P.a.*/UR, and 1% for *P.a.*/WE. The slight difference between males and females in P.a./MR was not significant at any level (t = 0.18).

Sexual dimorphism of contact chemoreceptors thus seems to be the rule in *Periplaneta americana,* with the strain *P.a.*/MR being an exception. The other species, however, lack sexual dimorphism in this character. All the strains of *Periplaneta americana* examined in this study were obtained from longstanding laboratory cultures, but changes in culture are no more likely an explanation of the observed variations than possible differences in the natural populations from which the cultures were derived. It seems most prudent at this point to keep these data in mind during future behavioral experiments, rather than drawing conclusions based on morphological data alone. Antennal contact between males and females has been found to play a role in the courtship behavior of several species of cockroaches (Roth and Barth, '67), although it has not been stressed in behavioral studies of *Peri-*
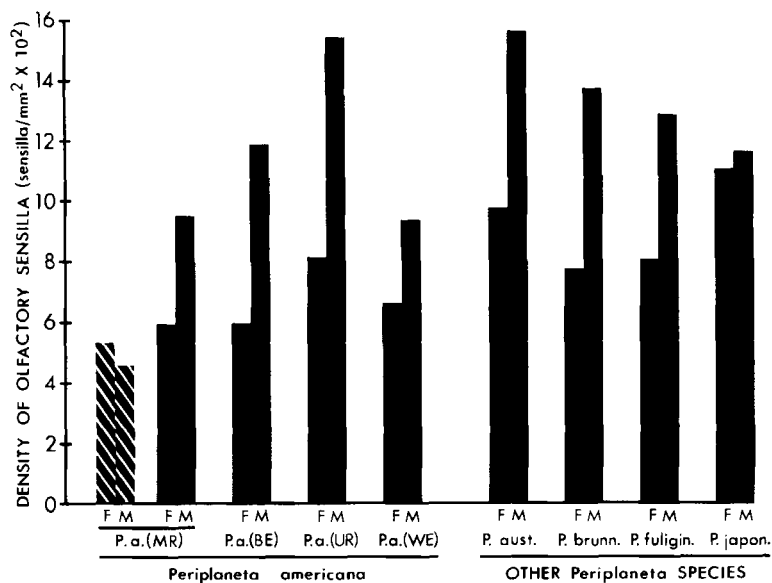
Fig. 8 Density of olfactory sensilla on the antennae of four strains of *Periplaneta americana* and four other species within the genus *Periplaneta*. Notations as in figure 3.

planeta americana (Barth, '61, '70; Simon, '71).

Observations on sensillar permeability

The use of crystal violet as an indicator of cuticular permeability (Slifer, '60) is a widely-applied and reliable method. We compared the pattern of staining with crystal violet to staining with $AgNO_3$ and $CoCl_2$ and found some potentially useful differences.

Crystal violet stained the porous tips and lumina of the contact chemoreceptors in *Periplaneta spp*. It also uniformly stained the distal two-thirds of the shafts of the olfactory sensilla. Silver nitrate stained the tips and distal lumina of the contact chemoreceptors (figs. 11, 18). In contrast, cobalt stained the olfactory receptors deeply, as well as the tips of the contact chemoreceptors (figs. 12, 16, 17). The reasons behind the different staining properties of the two new methods are unknown, but might easily be due to the different exposure times and the considerable osmotic difference between 0.1 M $AgNO_3$ and 1.0 M $CoCl_2$. Washing with 10% acetone prior to

$CoCl_2$ or $AgNO_3$ treatment also may have had a differential effect. Hawke and Farley ('71b) found that the penetration of antennal chemoreceptors by lanthanum nitrate differed when acetone or chloroform were used prior to immersion in the stain.

In our hands, the cobalt method produced a deeper and more sharply demarcated stain than the crystal violet method. The cobalt stain was also found to have the additional advantage of persisting when the antenna is permanently mounted. Crystal violet tends to become diluted and disappear if any water remains in the preparation or if the antenna is mounted in Hoyer's solution for clearing. We suggest that the cobalt stain be tried prior to use of the scanning electron microscope in the examination of olfactory sensilla. Since the cobalt would be a suitable emitter of secondary electrons, with or without coating of the specimen, it might be used to visualize the distribution of the pore tubule system. It might also be useful to apply organic solvents or enzymatic treatment prior to cobalt staining.

The extrusion of antennal fluids under

pressure (as described under MATERIALS, METHODS AND NOMENCLATURE) served to substantiate the results produced by the other methods. When the antenna was squeezed, droplets of fluid appeared at the tips of the contact chemoreceptors; when the pressure was released, the droplets were often drawn back into the sensilla. Fluid was not expelled from the olfactory sensilla.

New findings on sensillar morphology

The individual types of antennal sensilla in the new species are morphologically identical to those described in *Periplaneta americana* (Schafer and Sanchez, '73). However, two new observations resulted from the use of the extrusion method:

1. *Contact chemoreceptor articulation.* The base of each contact chemoreceptor is set into a socket composed of flexible cuticle. When sufficient internal pressure is created by squeezing the antenna, the thick-walled hairs move outward from the antenna into a more erect position. The socket is constructed with a hinge on the proximal side, toward the base of the antenna. Since the socket is hinged on the proximal side, the other side of the socket — its distal side — must undergo an accordian-like movement when the hair is deflected outward. Observation of this movement disclosed stretching of a bulbous body attached to the distal side of the socket at the base of the hair. This could be the site of attachment of the mechanoreceptor dendrite and the mechanotransducer mechanism. This preparation offers an opportunity for study of the transducer mechanism. For example, the transmission electron microscope could be used to view the receptor process fixed under varying degrees of stretch.

2. *Cold receptor sensillum.* This type of sensillum, first reported by Loftus ('66), is usually found distal to the first row of contact chemoreceptors on the ventral side of every other segment in the distal third of the antenna (Schafer and Sanchez, '73). In the present study, crystal violet and cobalt staining seemed to indicate the existence of an open tip and a central lumen (figs. 17-19). Use of the extrusion method confirmed this when drops of fluid were expelled from the tips of the cold receptor hairs but not from any portion of the olfactory sensilla. The structure of this type of sensillum is therefore quite different from that of the thin-walled olfactory sensilla, although they bear a superficial resemblance. Its structure is likely to be similar to that of coeloconic sensilla, and indeed, the cold receptor sensilla have a distribution which parallels that of the coeloconic sensilla on the antenna of the woodroach, *Leucophaea maderae* (Schafer, '71). Another line of evidence also indicates a basic difference between the cold receptor sensillum and the thin-walled olfactory sensilla. One antenna subjected to cobalt staining had some segments totally lacking olfactory sensilla. On these segments, however, cold receptor sensilla were found in the appropriate locations (fig. 19). This accident of development indicates that the cold receptors differentiate from different progenitor cells than the olfactory receptors. The contact chemoreceptors also develop from a different progenitor population than the olfactory receptors, because they were present on the segments which lacked olfactory sensilla. This suggests that the different sensory modalities on the antenna differentiate from different progenitors, except in the case of the thick-walled hairs which are bimodal, and subserve both contact chemoreception and mechanoreception.

The role of sexual dimorphism

Sexual dimorphism in the distribution of contact chemoreceptors and olfactory sensilla undoubtedly plays an important role in sexual behavior. In male *Periplaneta,* the full wing-raising display and turning or presentation are released by contact with the female (Barth, '70). The additional thick-walled hairs on male antennae would increase the intensity of stimulation derived from antennal contact. The extra hairs might also contain receptors special-

ized for contact pheromone reception. However, additional contact chemoreceptors are not absolutely necessary to perform this function in the male. This is illustrated by the lack of any male-female difference in the number of contact chemoreceptors in one of the four species of *P. americana* and in the other species examined.

Male olfactory sensilla are also likely to contain specialized receptors, since the airborne pheromone releases a specific, stereotyped behavior (antennal waving, rapid oriented locomotion, and partial wing-raising). It is therefore reasonable to attribute sexual dimorphism of olfactory sensilla in the antennae of *Periplaneta* to the existence of one or more populations or pheromone receptors in male antennae, but not in larvae or female adults. The nature of olfactory receptor specialization will be covered in another paper describing the electrophysiology of the *Periplaneta* antenna.

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PLATE 1

EXPLANATION OF FIGURES

9 Distal region of a segment on the antennal flagellum of an adult male *Periplaneta americana*. The olfactory sensilla are the thin-walled hairs or pegs. The olfactory sensilla are generally innervated by two sensory neurons. × 750.

10 Distal region of a segment on the antennal flagellum of an adult male *Periplaneta americana*. The fluted shaft of a thick-walled hair is visible emerging from its socket on the surface of the segment. The socket contains a hinge on its proximal side, toward the base of the antenna. × 750.

11 Three thick-walled hairs on adjacent segments in the proximal part of the antennal flagellum of *Periplaneta americana*. The tips and distal lumina of the hairs have been stained using silver nitrate. × 125.

12 A thick-walled hair on a segment in the antennal flagellum of *Periplaneta americana*. The tip and distal lumen of the hair have been stained with cobalt. This method also stains the porous areas of the olfactory sensilla. × 200.
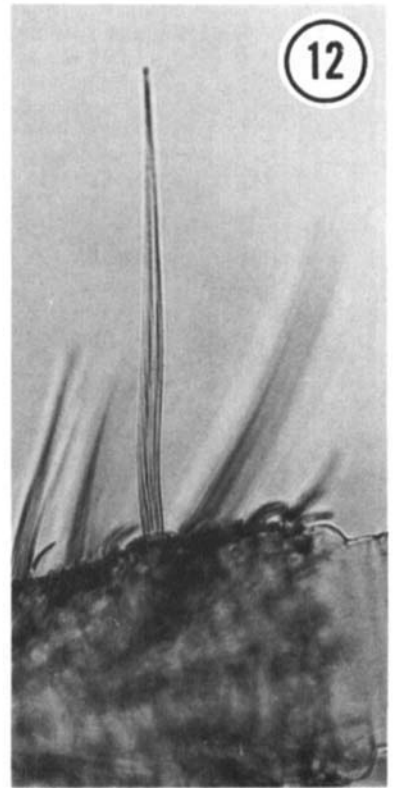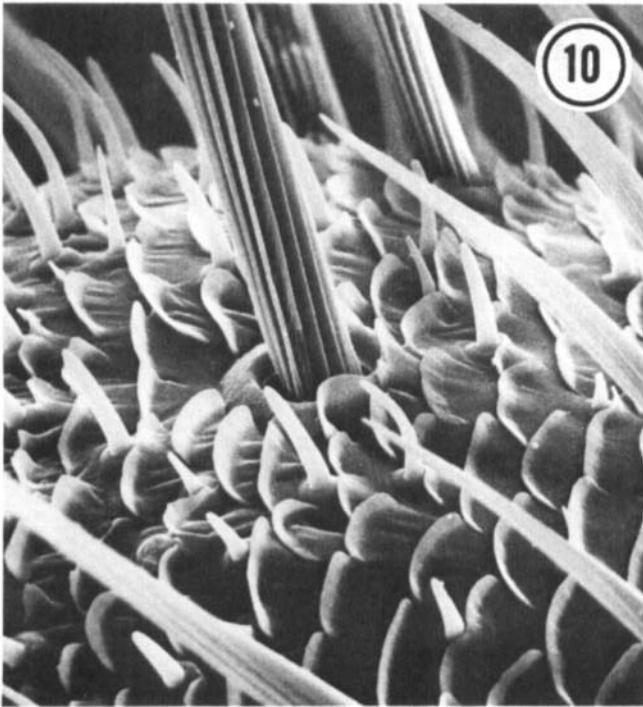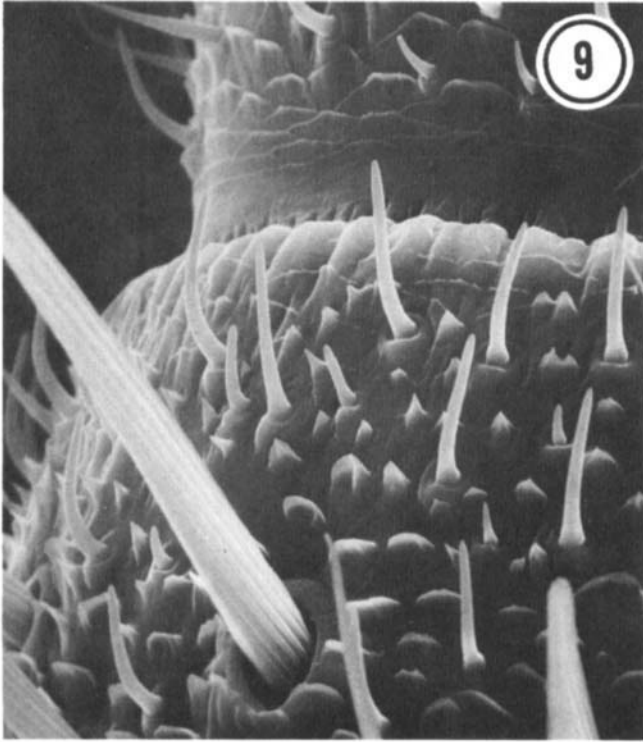
PLATE 2

13   Distal tip of a thick-walled hair. The tips of the thick-walled contact chemoreceptors are porous to crystal violet, silver nitrate, and cobalt chloride. In this scanning electron micrograph the porous area appears as a dark spot at the tip of the hair. This is the base picture (0° angle) for the stereographic representation of figures 14, 15. × 10,000.

14, 15   Stereographic representation of the tip of a thick-walled hair, ± 2.5°. The dark area at the tip of the hair appears to be a raised area with a different composition than the cuticle of the hair shaft. The stereograph may be viewed with a stereoscopic viewer (e.g., CF-8 Stereoscope, Abrams Instruments, Lansing, Michigan). The horizontal streaks in these micrographs are artifacts which result from electrical charging of the hair induced by the electron beam. × 10,000.

PLATE 3
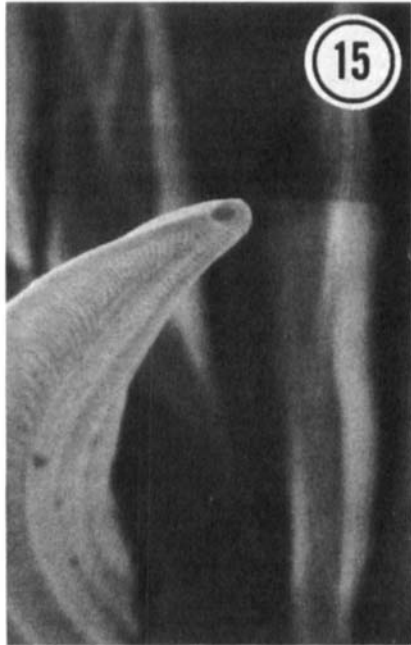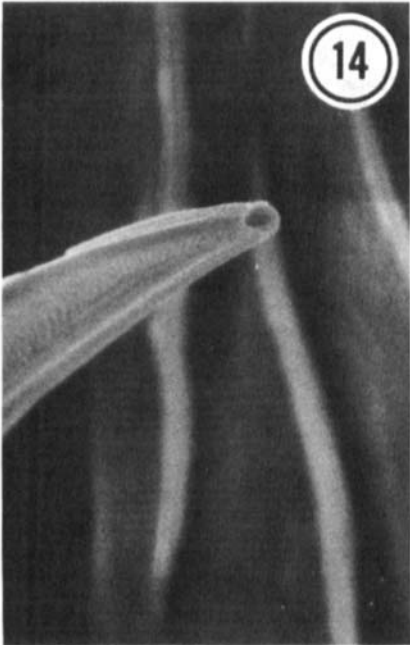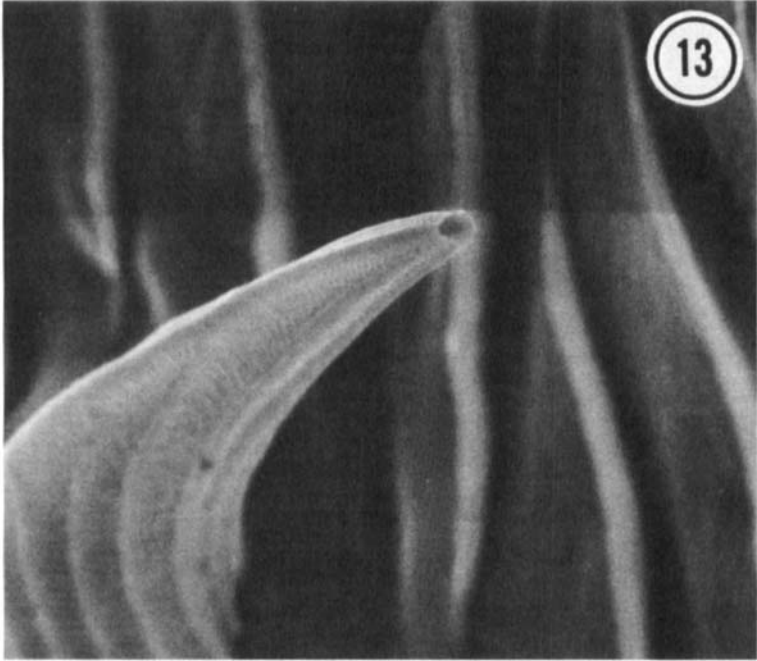
16 Distal segment of an antenna of *Periplaneta americana* stained with cobalt chloride. Cobalt has penetrated the porous areas of the thin-walled hairs, resulting in the darkening of the distal one-third to one-half of the hair shaft. Cobalt has also penetrated the tips of the thick-walled hairs, although this is not readily visible owing to the plane of focus. × 125.

17 Cobalt-stained olfactory sensilla on the distal margin of a segment in the antennal flagellum of *Periplaneta americana*. The distal one-third to one-half of the cuticle of the thin-walled hairs has been stained. This staining probably corresponds to the location of the pore-tubule system. A cold receptor sensillum (arrow) is also visible. × 200.

18 Cold receptor sensillum (of Loftus, '67) near the distal margin of a segment in the antennal flagellum of *Periplaneta americana*. The cold receptor sensillum indicated by the arrow has been stained with silver nitrate. Silver nitrate has entered the tip of the sensillum and has partially stained a central lumen. Thin-walled olfactory sensilla are also visible surrounding the cold receptor, but have not been stained by the silver nitrate. This sensillum is probably homologous to the coeloconic sensilla which have an identical distribution on the antennae of *Leucophaea maderae* (Schafer, '71). × 200.

19 Single cold receptor sensillum on a segment in the antennal flagellum of *Periplaneta americana* which lacks olfactory sensilla. In this rare specimen, the olfactory sensilla have failed to differentiate on some of the flagellar segments. Cold receptor sensilla were found in their normal distribution on this antenna, even though about a third of the segments lacked olfactory sensilla. This segment is from the same antenna illustrated in figure 18. × 200.