

EXTERNAL ANTENNAL MORPHOMETRY OF *TRICHOPLUSIA NI* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE)*

M. S. MAYER, R. W. MANKIN† and T. C. CARLYSLE

Insect Attractants, Behavior and Basic Biology Research Laboratory, Agricultural Research, Science and Education Administration, USDA Gainesville, FL 32604, U.S.A.

(Accepted 5 November 1980)

Abstract—A detailed morphometric analysis of the antennae of male and female *Trichoplusia ni* (Hübner) is presented, based on light and scanning electron microscopic observations. These measurements of general antennal dimensions and the number, distribution, dimensions, and surface features of the antennal sensilla are applied in the consideration of olfactory transduction processes and in the derivation of a mathematical representation of the antennal surface. The surface area and the number of sensilla on the antennal flagellum are found to vary with the weight of the pupa and adult moth. The long Type I sensilla trichodea are hypothesized to be innervated by pheromone-sensitive olfactory cells on the basis of morphological comparisons with sensilla of other insects.

Index descriptors (in addition to those in title): Sensilla, trichodea, numbers, proportions, pores, pore-surface area.

INTRODUCTION

THE MODERN concepts of olfactory transduction and olfactory thresholds in insects are based on exacting measurements of the number, dimensions, and proportions of the different olfactory sensilla on the antenna, as well as the number and distribution of pores on the sensillar surface (Boeckh *et al.*, 1965; Adam and Delbrück, 1968; Kaissling, 1971). The calculations of olfactory thresholds are based primarily on studies of *Bombyx mori* (L.) (Kaissling and Priesner, 1970), which has a plumose antenna. It is not immediately obvious how to apply these calculations to insects with filiform antennae such as the cabbage looper, *Trichoplusia ni*, because of the large differences in sensillar number and antennal surface area.

The cabbage looper has been the subject of extensive investigations of pheromone-induced behavior; thus, it is a logical choice for complementary studies of pheromone, transduction processes and electroantennogram (EAG) characteristics. In preparation for such studies, currently in progress, we have examined the antennal surface of *T. ni* in detail to obtain estimates of critical morphological parameters. These parameters, including the general dimensions of the antenna, the number and distribution of sensillar types, and the dimensions and surface features of the sensilla, are also of interest from a developmental and evolutionary viewpoint. A unique contribution of this study to the understanding of antennal morphology is that we have derived a mathematical model of

*Mention of a commercial or proprietary product in this paper does not constitute an endorsement of that product by the USDA.

†Postdoctoral fellow, employed through a cooperative agreement between the Department of Entomology & Nematology, University of Florida, and the Insect Attractants, Behavior, and Basic Biology Research Laboratory, AR/SEA, USDA, Gainesville, FL 32604, U.S.A.

sensillar number and distribution that takes into account the variations in adult and antennal sizes. The mathematical representation is useful, for example, in understanding the relationship between the EAG potential and the fraction of the antennal surface exposed to an odorant (Mayer, Mankin and Lemire, unpublished).

Zacharuk (1980) and Altner (1977) have attempted to correlate morphology with function and to apply terminology that stresses this relationship. For example, Zacharuk classifies all olfactory sensilla into one category of "multiporous chemosensilla" with either pitted or grooved surfaces. However, the older terminology was used in earlier work with *T. ni* (Jefferson *et al.*, 1970; Lin and Chow, 1972), and we have continued this usage to simplify comparisons between the 3 reports.

PROCEDURES

Antennae from two different groups of adult *T. ni* were studied. One group, comprising 7 males and 7 females, was selected from laboratory-reared pupae weighing 180–200 mg. One antenna from each moth was examined by both light and scanning electron microscopy (SEM) (Cambridge, Mark IIA) to determine the subsegment length and diameter as well as the number, dimensions, and proportions of the different sensilla on the surfaces of subsegments 1, 5, 10, 15, . . . , 75, counting from the base. The specimens analyzed by SEM were freshly excised antennae mounted on stubs with either silver-based paint (DuPont®, conductive silver) or double stick tape and coated with gold in either a high vacuum evaporator (Denton Model DV-502) or a sputter coater (Hummer Model II). The specimens studied by light microscopy were mounted between 2 microscope cover slips in CMC-10 (Turtox, Chicago, IL).

The three types of sensillum trichodea on the antennae (Jefferson *et al.*, 1970) were counted as follows: Type III, 6 antennae with the light microscope and 4 with the SEM; Type II, 8 antennae with SEM; and Type I, 5 antennae with the light microscope. The number of sensilla trichodea per unit surface area was measured using light microscopy by focusing a calibrated 20 × 20 ocular grid at a level just below the surface of the cuticle of antennal whole mounts. At this level the bases of the sensilla trichodea show up brighter than the background as point sources of light. The density of sensilla trichodea was measured from each 5th subsegment on one antenna from 5 males and 5 females.

Counts of sensilla from areas as large as 3300 μm² were possible from the proximal subsegments. Distally, relatively more antennal sensory surface is covered by the sensilla coeloconica and the tracts of sensilla trichodea become progressively smaller. Thus, on the more distal subsegments (No's. 55 and up), it was common to have to count the sensilla from areas of not more than 1400 μm².

The second group of moths, comprising 10 males and 8 females, was selected from laboratory-reared pupae weighing 120–300 mg. This increased range of weights was chosen to allow correlation of pupal weight with antennal surface area. One antenna from each moth was examined by light microscopy. The length and diameter of subsegments 1, 5, 10, 15, . . . , 75 were measured with a calibrated ocular micrometer.

We calculated subsegment surface areas and volumes for the antennae of the moths in both weight groups from the equations:

$$S = \pi dl \quad (1)$$

and

$$V = \pi d^2 l / 4, \quad (2)$$

where S (μm²) is the subsegment surface area, d (μm) is the mean subsegment diameter, l (μm) is the mean subsegment length and V (μm³) is the subsegment volume. The length, volume, and surface area of the entire flagellum were calculated by summing over 75 subsegments. In addition, polynomial (n th-order) regressions of subsegment diameter and length, as well as the number and proportion of sensilla on each subsegment, were calculated as functions of subsegment number. The general form of the regression is

$$Y = b_0 + b_1x + b_2x^2 + b_3x^3 + b_4x^4 + \dots + b_mx^m, \quad (3)$$

where Y is the parameter to be estimated, $b_0, b_1, b_2, b_3, \dots, b_n$ are regression coefficients, and x is the number of subsegments from the base.

RESULTS

We first consider the general dimensions of the antenna and then examine in detail the number and types of sensilla on the antennal surface of the 180–200 mg moths. Three

typical subsegments are diagrammed in Fig. 1 to indicate both the diversities and similarities of the subsegments encountered in various regions of the flagellum. No sensilla trichodea were found on the scape and pedicel, although several fields of bristles described by Böhm (1911) occur on these segments. Sensilla trichodea generally appear first on subsegment 3, occasionally on subsegments 1 and 2, and their density on this subsegment was more variable than on any of the next few subsegments.

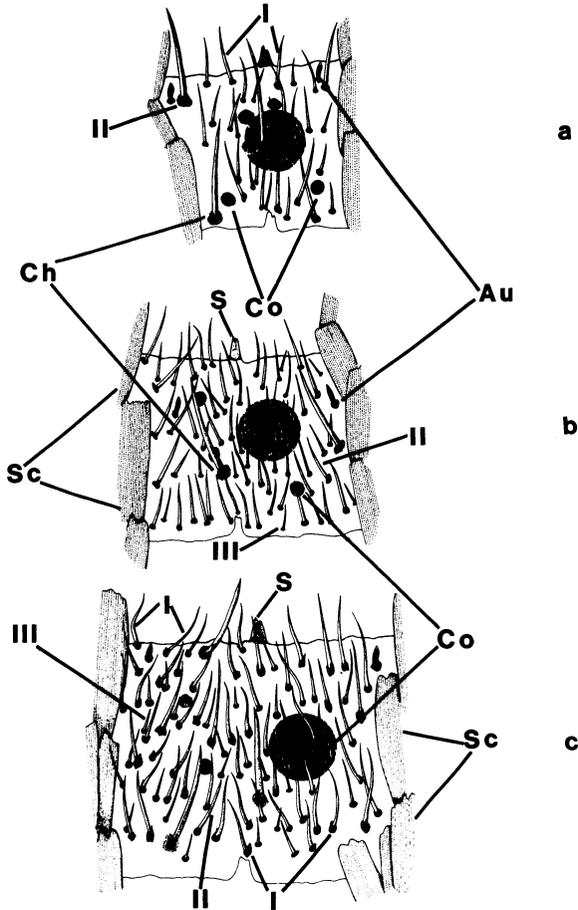


FIG. 1. Diagram of typical antennal subsegments from (a) distal, (b) medial, and (c) proximal locations on the flagellum of the cabbage looper. Surface sculpturing is represented within circular areas. Representative sensilla are: Au, sensilla auricillica; Ch, sensilla chaetica; Co, sensilla coeloconica; S, sensilla styloconica; I, sensilla trichodea, Type I; II, sensilla trichodea, Type II; III, sensilla trichodea, Type III; and Sc, scales.

Flagellum and subsegment dimensions

The measurements of subsegment diameter and length from the 180–200 mg moths indicated that the diameter decreased progressively from the base to the last subsegment and the length increased from the base up to subsegment 15, after which it decreased rapidly. The regressions of diameter and length on subsegment number are listed in Table 1. The subsegment areas calculated from equation 1 are shown in Fig. 2. There was no

TABLE 1. COEFFICIENTS OF BEST FIT FOR THE REGRESSIONS OF MORPHOMETRIC DATA*

	b_0	b_1	b_2	b_3	b_4	$r^{2\dagger}$	$p(H_0) \leq \dagger$
Subsegment dimensions							
diameter (d) (μm)	154.4259	-0.8785	-0.0036	-	-	0.887	0.01
length (l) (μm)	141.3668	0.8017	-0.0194	-	-	0.879	0.01
Antennal surface area (mm^2)§	-2.615	0.008	-	-	-	0.524	0.05
Total number of sensilla trichodea (N)	-34.60725556	23.83318776	-0.93729626	0.01401436	-0.00007389	0.977	0.0001
Proportions of sensilla trichodea:							
Type I (P_I)	♂	-0.02575705	0.00051138	-0.00000350	-	0.989	0.001
	♀	-0.02763764	0.00064623	-0.00000451	-	0.889	0.001
Type II (P_{II})	♂	-0.02269750	0.01686343	-0.00036500	0.00000255	0.961	0.001
	♀	0.10557566	0.01445606	-0.00034824	0.00000243	0.799	0.001
Type III (P_{III})	♂	-0.02217672	0.00889272	-0.00014557	0.00000094	0.990	0.001
	♀	0.00762084	0.00488756	-0.00007982	0.00000042	0.938	0.001
Sensilla auricillica number (N)	♂	0.16045767	0.08847503	-0.00087072	-	0.935	0.0001
	♀	0.11450778	0.10709735	-0.00107976	-	0.872	0.0001
Sensilla coeloconica number (N)	♂	0.21136340	0.15237077	-0.00129543	-	0.969	0.0001
	♀	0.48135404	0.20637487	-0.00178648	-	0.948	0.0001
Sensilla chaetica number (N)	♂	1.27582418	0.09487123	-0.00046839	-	0.970	0.0001
	♀	0.49637139	0.16356513	-0.00138046	-	0.937	0.0001
length (l) (μm)	41.62813187	0.82006884	-0.00822140	-	-	0.918	0.0001
Modeled number of sensilla trichodea (N)	-21.78056490	20.13234499	-0.72917819	0.00992586	-0.00004784	0.986	0.0001

*Except where noted the independent variable is subsegment number (see equation 3).

†Coefficient of determination.

‡Probability that b_1 and higher order terms are zero.

§Independent variable is adult weight in mg.

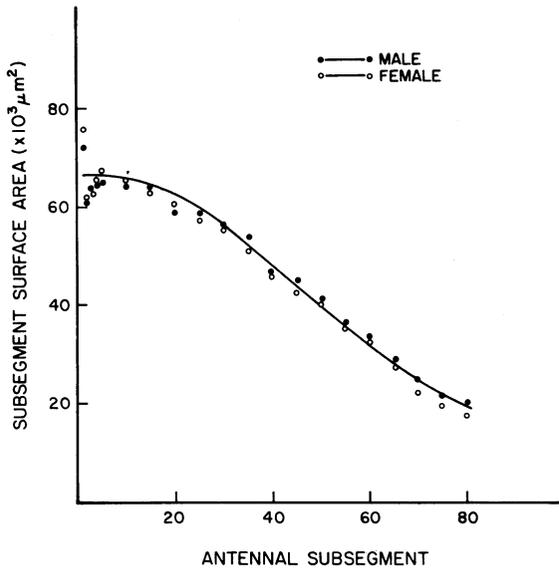


FIG. 2. Subsegment surface area of male and female cabbage loopers weighing 180–200 mg (estimated surface area = $65.93291418 + 0.12347001X - 0.01893612X^2 + 0.00012436X^3$; $r^2 = 0.984$, $p(H_0) < 0.01$).

difference between the total surface area of antennae from males (3.6 mm^2 ; 95% confidence interval = 0.4 mm^2) or females (3.6 mm^2 ; 95% confidence interval = 0.3 mm^2) calculated by summing the projected surface areas from 75 subsegments. Steinbrecht (1970) estimated that surface sculpturing increases the area of the antennal flagellum of *B. mori* 3–4 times. If the same is true of *T. ni*, we would expect that each flagellum would have a total surface area of about $10\text{--}15 \text{ mm}^2$. The projected sum of the 75 subsegmental lengths indicated that the total flagellar length for adults from 180 to 200 mg pupae was 1.01 cm, a length 25% shorter than the length of the *T. ni* antennae studied by Jefferson *et al.* (1970). We have no explanation for this discrepancy and can only surmise that strain differences are involved. Although the calculated surface area provides a smooth curve, the first 5 subsegments were always more variable than any other subsegments of the antenna. Subsegment 2 was invariably shorter than either subsegments 1 or 3, and subsegment 1 had a larger surface area than any other subsegment on the antenna (Fig. 2).

Relationship of pupal and adult weight and antennal surface area

The first moths examined were all from pupae that weighed from 180 to 200 mg, but there appeared to be differences in the total number of sensilla trichodea on particular antennae. As the studies progressed, we adduced that the larger the antenna, the more numerous the sensilla. To determine if this affect was due solely to the increase in surface area, we examined the relationship between adult weight and antennal surface area and the relationship between pupal weight and antennal surface area.

The relationship between pupal weight and antennal surface is illustrated in Fig. 3. There was an increase in area of about 0.007 mm^2 for each mg of pupal weight. Also, there was a significant correlation between the weight of the adult and the total surface

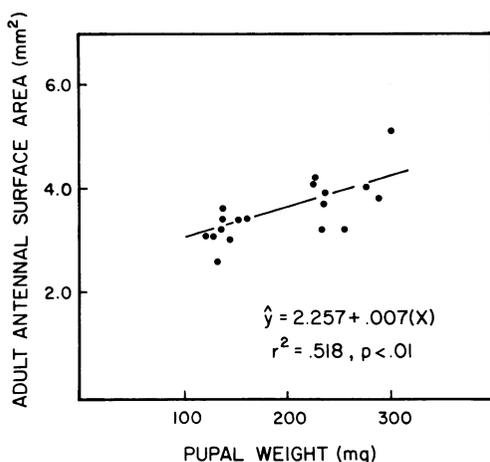


FIG. 3. Relationship of pupal weight to total surface area of antennal flagellum of cabbage looper.

area of the flagellum (see Table 1). Each mg of adult weight is translated into 0.008 mm^2 of added antennal surface area.

Dimensions of trichoid sensilla

Jefferson *et al.* (1970) classified the three types of sensillum trichodea on the antenna as Types I, II and III (Fig. 1). These are all "multiporous, thin-walled, olfactory chemosensilla" according to the terminology of Zacharuk (1980). Our criteria for distinguishing them were as follows. Type I: these are generally the longest of the sensilla, and are about $2.0-2.5 \mu\text{m}$ in diameter at the base; they taper gradually into a very sharp point at the distal ends. Spiral annulations gird the entire length of the sensilla with small pore funnels opening ($\geq 100\text{\AA}$) between the annulations. Type II: these sensilla are distinguishable from Type III sensilla only by length, i.e., their minimum length is generally greater than the maximum length of Type III sensilla over most of the antennal flagellum. This difference could be distinguished more easily by light microscopy than by SEM. Both Type II and III sensilla arise from bases $2.0-2.5 \mu\text{m}$ in diameter, but unlike Type I sensilla, they retain for the most part their original diameter, ending distally in a rounded tip. In both Type II and III sensilla, pore funnels are confined to spiraling rows that converge on a junctional area extending the length of the sensillum.

The length of two or three sensilla trichodea was measured on each 5th subsegment from three antennae each of the males and females. The data are summarized in Fig. 4. The length of the Type I sensilla is maximal around subsegment 15-25 on males and females. The average lengths of the Type II and III sensilla remain about the same over the length of the flagellum. There was some overlap in the range of lengths of Type II and III sensilla. The composite average lengths, 95% confidence interval, and range of lengths are:

	Type I	Type II	Type III
Males: mean \pm conf. int.	40.4 ± 1.7	23.4 ± 1.0	15.1 ± 0.5
range	28-58	18-34	12-19
Females: mean \pm conf. int.	41.6 ± 1.3	27.4 ± 1.0	17.3 ± 0.9
range	25-58	15-42	9-26

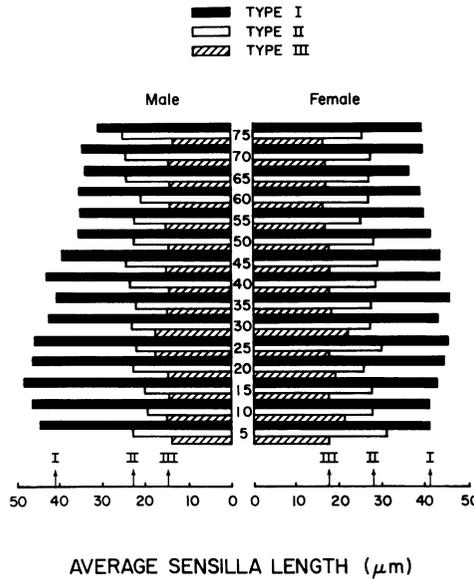


FIG. 4. Average lengths of sensilla trichodea on antennae of male and female cabbage loopers. Arrows on horizontal scale are placed at average length of sensilla types.

Number and density of trichoid sensilla

The number of sensilla trichodea varied with the size of the antenna. The simplest approximation to the actual distribution was to assume that the density of sensilla and the proportion of different types of sensillum on a given subsegment remained constant across different antennae. This allowed us to model the actual number of sensilla on a subsegment as being proportional to subsegment surface area. Consequently, when we counted the number of sensilla, we also determined both the sensillar density and the proportion of different sensillar types on subsegments 1, 5, 10, 15, . . . , 75 on antennae of adults reared from the 180 – 200 mg pupae.

The counts of sensilla trichodea are shown in Fig. 5. The number of sensilla increased

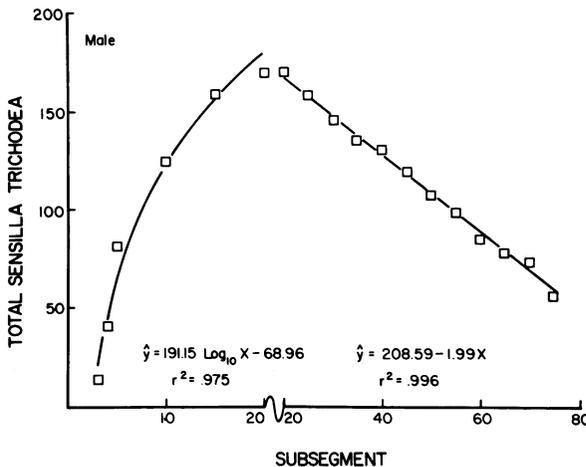


FIG. 5. Distribution of sensilla trichodea on flagellum of males.

logarithmically with subsegment number from subsegments 3 – 20; thereafter, the number decreased in a linear fashion. The curvilinear regression of best fit is listed in Table 1. The two curves in Fig. 5 were obtained by separating the counts into two groups comprising subsegments 3 – 20 and 21 – 75.

The density of sensilla trichodea was obtained by a series of measurements on subsegments 3 – 10, 12, 14, 16, 18, 20, 25, 30, . . . , 75. Up to subsegments 10 – 12, the density increased with subsegment number (Fig. 6). Afterwards, it remained constant. These measurements do not confirm statements concerning the spacing (and consequently the density) of the sensilla past subsegment 10 on the cabbage looper antenna by Callahan (1975; Fig. 8, p. 397).

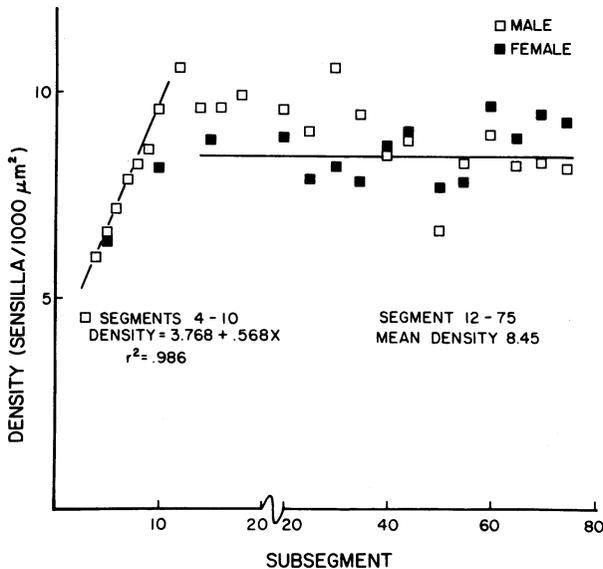


FIG. 6. Density of sensilla trichodea on antennal subsegments.

Proportions of Type I sensilla trichodea

Type I sensilla sometimes were observed on subsegments 1 and 2 in the female but never on subsegment 1 in the male. Thus, there was a slight difference in the distribution of Type I sensilla in males and females, but there was no real difference in the total number. The proportion of Type I sensilla on the female antenna levels off around subsegment 20; in males it continues to decrease (Fig. 7). The curvilinear regressions of best fit for the proportions of Type I sensilla were 3rd order polynomial expressions listed in Table 1.

Proportions of Type II sensilla trichodea

The proportion of Type II sensilla varies somewhat along the subsegments of the antenna and is slightly different in males and females. In males the proportion increases up to about subsegment 40 and levels off thereafter (Fig. 7). The proportion of Type II sensilla on female antennae is greater than on the male and is maximal at about subsegment 30; decreasing thereafter until it eventually has about the same proportion as the male at subsegment 75. As with the Type I, the curvilinear regressions of best fit for

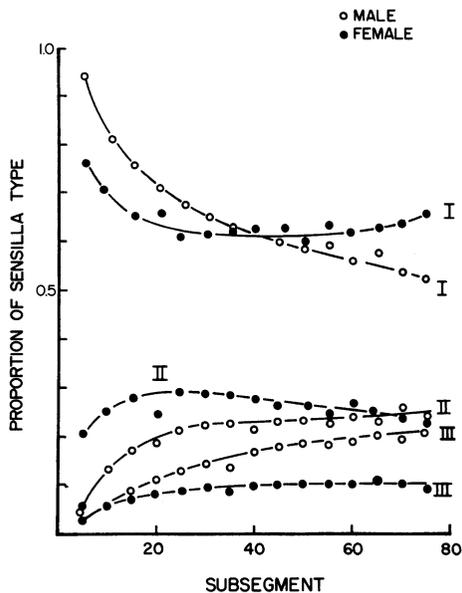


FIG. 7. Proportions of 3 types of sensillum trichodea on flagellum.

the proportions of Type II sensilla were 3rd order polynomial expressions. The regression coefficients are listed in Table 1.

The pores in the cuticle of the sensilla are the sites of entry for odorant molecules (Kaissling, 1971), and some quantitative estimates of the number and density of pores on the sensilla of other insects have been made (Schneider and Steinbrecht, 1968; Kaissling, 1971). In *T. ni* the pores open from funnels of $\geq 1000\text{\AA}$ diameter. The density of pores in Type II and III sensilla from male and female antennae was measured directly from SEM photographic montages at X22,000. Image foreshortening was avoided by adjusting the specimen so that the sensilla lay horizontally in the beam. At least 2 estimates of pore density were obtained from at least 2 sites on each sensillum (Table 2). Only small differences in pore density were observed. Twenty-four measurements yielded an average 37 pores/ μm^2 , with a range of 34–44 pores/ μm^2 . Because the average surface area of a Type II sensillum trichodea is about 160 μm^2 , we estimate a total of about 5920 pores/sensillum.

Proportions of Type III sensilla trichodea

The Type III sensillum trichodea, like the Type II, is a multiporous, thin-walled sensillum that probably has an olfactory function. Length is less variable than the length of the Type I and II sensilla. There are more Type III sensilla on the male than on the female antenna (Fig. 7, Table 2). The curvilinear regressions of best fit for the proportions of Type III sensilla are listed in Table I.

The pore density for Type III sensilla was measured in the same way as for the Type II sensilla. The pore densities and locations in the males and females were identical to those for the Type II sensilla. There were 37 pores/ μm^2 , thus, there are a total of about 3700 pores/Type III sensillum.

TABLE 2. SUMMARY OF MORPHOLOGICAL DATA RELATING TO THE ANTENNA OF *Trichoplusia ni*

Sensillum type	Total number of sensilla per antenna	Percentage of total	Average length (μm)	Diameter (μm)	Total surface area/antenna (μm^2)	Total pores/sensillum	Total pores/antenna	Total volume/antenna (μm^3)
Males								
Trichodea								
I	5417	60.1	$40.4 \pm 1.7^*$	2.0	1.4×10^6	806†	$4.4 \times 10^{6\dagger}$	6.9×10^5
II	1683	18.7	$23.4 \pm 1.0^*$	2.0	2.5×10^5	5440	9.3×10^6	1.2×10^5
III	1149	12.7	$15.1 \pm 0.5^*$	2.0	1.1×10^5	3510	4.1×10^6	5.5×10^4
Coeloconica	264	2.9	—	7.0‡	1.0×10^4	—	—	—
Pit opening								
Peg			4.0‡	2.0‡	6.6×10^3	—§	—§	3.3×10^3
Auricillica	139	1.5	14.8‡	2.8‡	1.8×10^4	4817	6.7×10^5	1.3×10^4
Styloconica	65	0.7	$19.3 \pm 0.8^*$	$5.6 \pm 0.2^*$	2.2×10^4	1-2	80+	3.2×10^4
Chaetica	300	3.3	$54.6 \pm 1.5^*$	$3.1 \pm .06^*$	1.7×10^5	1	300	5.2×10^5
Total	9017							
Females								
Trichodea								
I	5400	58.8	$41.6 \pm 1.3^*$	2.0	1.4×10^6	829†	$4.5 \times 10^{6\dagger}$	7.1×10^5
II	2169	23.6	$27.4 \pm 1.0^*$	2.0	3.7×10^5	6370	1.4×10^7	1.9×10^5
III	730	7.9	$17.3 \pm 0.9^*$	2.0	7.9×10^4	4022	2.9×10^6	3.9×10^4
Coeloconica	368	4.0	—	7‡	1.4×10^4	—	—	—
Pit opening								
Peg			4‡	2‡	9.2×10^3	—§	—§	4.6×10^3
Auricillica	159	1.7	14.8‡	2.8‡	2.1×10^4	4817	7.7×10^5	1.4×10^4
Styloconica	65	0.7	$19.3 \pm 0.8^*$	$5.6 \pm 0.2^*$	2.3×10^4	1-2	80+	3.2×10^4
Chaetica	300	3.3	$56.9 \pm 1.3^*$	$3.1 \pm .06^*$	1.7×10^5	1	300	5.2×10^5
Total	9191							

*95% Confidence interval.

†Based on a measurement of 3.17 pores/ μm^2 , from a photograph by R. J. O'Connell, Worcester Foundation (unpublished).‡From Jefferson *et al.* (1970) and Mayer (unpublished).

§Unable to estimate.

Sensilla auricillica

This sensillar type was so named by Jefferson *et al.* (1970). Sensilla auricillica are thin-walled, multiporous sensilla innervated by several olfactory receptor cells. They are predominantly located at the laterodistal margins of the sensillar field on each subsegment. Very rarely was one found more proximally, but none were observed away from the lateral margin of the subsegment. Also, none were observed on either of the first 2 subsegments, but they were common on the third. No more than 4 were ever observed on any one subsegment of either males or females, and 2 or 3 was the more common total. The curvilinear regressions of best fit for the number of sensilla auricillica on male and female antennae are listed in Table 1 and plotted in Fig. 8. The 2 regressions project a total of 139 and 159 sensilla auricillica on the 75 subsegments of male and female antennae, respectively. These values were confirmed by actual counts and agree with those reported by Jefferson *et al.* (1970).

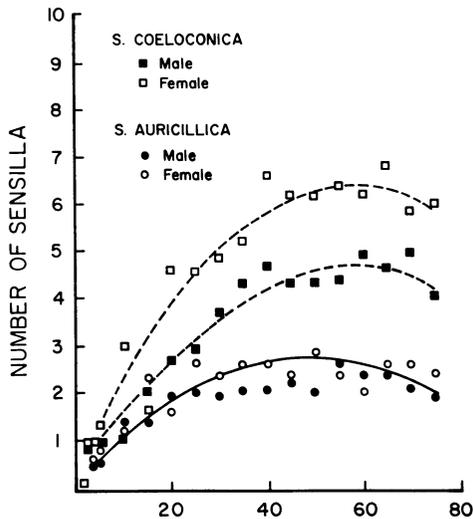


FIG. 8. Distribution of sensilla coeloconica and sensilla auricillica along flagellum.

The sensillum auricillica resembles a cylinder with a concave indentation. The surface area calculated for an individual sensillum was $130 \mu\text{m}^2$. The volume was $91.1 \mu\text{m}^3$. The pore density is not significantly different from that for Type II or III sensilla trichodea; thus, an individual sensillum auricillica has about 4817 pores (Table 2). However, we were unable to determine if pores are found on the undersurface.

Sensilla coeloconica

The morphology of this type of sensillum was described by Jefferson *et al.* (1970). Sensilla coeloconica sometimes are found on subsegment 1 of female antennae, but not proximal to subsegment 3 of male antennae, though where they first arise is variable. The distribution of this sensillum is discontinuous on any one antenna, but it can be modeled for a group of flagella by using 2nd order polynomial regression equations (see Table 1 and Fig. 8). The equations project a total of 264 and 368 coeloconica on the 75 subsegments of male and female antennae (Table 2), respectively. There is a discrepancy

in the total number of coeloconica estimated in this study and that previously reported by Jefferson *et al.* (1970), an average of 1–2 sensilla/subsegment on the male antenna and of 0–1 on the female antenna. Although the distribution of the sensilla is discontinuous among individual animals this difference suggests that there may be some genetic differences between the two groups of insects studied.

Sensilla chaetica

The sensilla chaetica are heavily sclerotized annulate sensilla terminating in a single apical pore. These structures arise from a movable membranous socket and are discontinuously distributed along the length of the flagellum. Neurophysiological recordings have confirmed the expected mechano-sensory function from apparently a single neuron. No other sensory cells of other functionality were observed. The distribution of sensilla chaetica on the male antenna is as follows: up to about subsegment 10, there are 2 sensilla chaetica located medially on the ventral side and one on the dorsal side; from about subsegments 25–35 another sensillum chaetica is added to the ventral surface; at about subsegment 40 a second sensillum chaetica is added to the dorsal surface; and finally, at about subsegment 45–50 a fourth sensillum chaetica is added to the ventral surface. On any particular subsegment there may be one more or one less sensillum chaetica than described. Generally, the sensilla are evenly distributed between the ventromedial, dorsomedial and ventrolateral surfaces. The female antenna is slightly different in that the second dorsomedial sensillum chaetica is rarer than in males and that the addition of an extra sensillum chaetica is less discontinuous than in males.

The curvilinear regressions of best fit for the distribution of sensilla chaetica on an average group of male and female antennae were 2nd order polynomial expressions listed in Table 1. The regressions predict a total of about 300 sensilla chaetica on 75 subsegments and 330 on 80, close to the number found by Jefferson *et al.* (1970). Because the antennae measured in these two separate investigations were materially different in length, and, consequently, in surface area, it is unlikely that the number of sensilla chaetica is determined by surface area.

The length and diameter of 4 sensilla chaetica from every fifth subsegment were measured from 4 females and 3 males. The average proximal diameter and 95% confidence interval were $3.13 \pm 0.06 \mu\text{m}$. There was no material difference in the lengths of the sensilla chaetica from males and females, and the length was maximal at about subsegment 50 and less at each end. The curvilinear regression of best fit for the length of sensilla chaetica on male and female antennae is listed in Table 1.

Sensilla styloconica

These sensilla appear identical to similar sensilla on the proboscis. Such sensilla have been shown to be contact chemoreceptors on the larval maxillary palps (Schoonhoven and Dethier, 1966). They were described by Jefferson *et al.* (1970). Only one of this type of sensillum is found mid-ventrally at the distal end of each subsegment beginning at about the eighth or ninth subsegment. Measurements show that the eminence on which the sensillum styloconica is located is shortest in the basal subsegments (10–14 μm), reaches its maximum length in the middle of the flagellum (about 24 μm), and decreases distally to a minimum of 14–18 μm .

The surface area and volume of the eminences on which these sensilla are found were computed from measurements of 3 males and 7 females. There was considerable

variation, but the eminences of the females sampled were always 2 μm longer than eminences of the males (Table 2).

DISCUSSION

It remains to consider the applications for which this study was originally designed. First we derive a model of the distribution of sensilla trichodea on the flagellum. Then we examine how the morphometric data and the model apply to olfactory transduction, particularly with respect to sex pheromone.

Modeling the total number of sensilla trichodea on the flagellum

To mathematically approximate the number of sensilla trichodea on a flagellum on a moth of any given weight, we assumed that the density of sensilla, the fraction of the surface occupied by sensilla, and the proportion of sensillar types on a given subsegment were constant for different sized antennae. The model equation is:

$$N = \sum_{x=3}^{75} f_x D_x S_x, \quad (4)$$

where N is the number of sensilla trichodea on the flagellum, x is the number of subsegments from the base, f_x is the fraction of subsegment x occupied by sensilla, D_x is the density of sensilla on subsegment x and S_x is the surface area of subsegment x . The summation begins at $x = 3$ because there are few sensilla on subsegments 1 and 2. The number of sensilla trichodea of a given type is:

$$N_i = \sum_{x=3}^{75} P_{i,x} f_x D_x S_x, \quad (5)$$

where $i = \text{I, II, or III}$, $P_{i,x}$ is the proportion of sensilla of type i on subsegment x , and N_i is the number of sensilla of type i .

Because the regressions for $P_{i,x}$, D_x , and S_x have already been determined (see Table 1), the solutions of equations 4 and 5 require merely an estimate for f_x . We were unable to measure this fraction directly because of the increasing proportion of antennal surface taken up distally by the sensilla coeloconica and other sensilla. Consequently, we adopted the indirect method discussed below.

Estimate of the proportion of the antennal area occupied by sensilla

The model estimate of f_x , the fraction of the surface area of subsegment x occupied by sensilla, was obtained from the equation

$$f_x = N_x / D_x S_x, \quad (6)$$

where N_x is the counted number of sensilla on subsegment x of antennae on adults reared from 180 to 200 mg pupae. The parameters D_x and S_x are defined in equation 5. Implicit in this equation is the assumption that the f_x measured for the 180–200 mg specimens remains constant for antennae of other surface areas. This fraction was found to increase logarithmically with subsegment number up to about subsegment 20 and to remain constant thereafter at about 0.32 (or 32%) (Fig. 9). Although visual inspection of the antenna gave us the impression that 50% of the subsegmental area is occupied by the

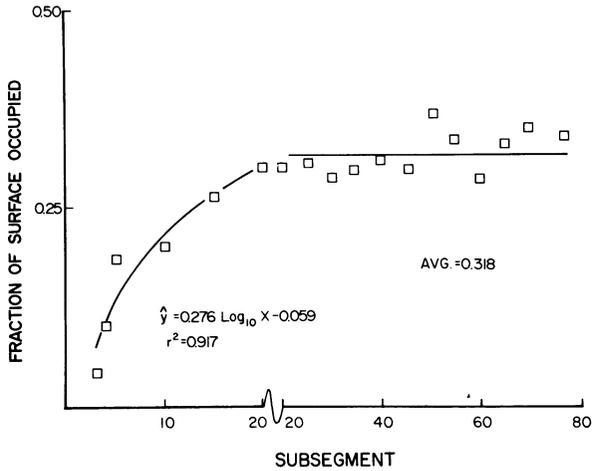


FIG. 9. Fractions of antennal subsegmental surface area occupied by sensilla.

sensory sensilla, especially at the distal end of the antenna, this amount was not confirmed by the calculation.

Predictions of the model

To demonstrate the application of the model, consider the predicted number of sensilla on subsegment 15 of the antenna from a 200 mg moth. The predicted surface area is $63.9 \times 10^3 \mu\text{m}^2$, the sensillar density is 8.45 sensilla trichodea/ $10^3 \mu\text{m}^2$ and the fraction of sensilla-occupied surface is 0.266, which results in a total of 143.6 sensilla trichodea. This compares with an actual counted average of 159.8 sensilla at segment 15 and 143.9 sensilla at subsegment 13 (Fig. 5). The fourth order regression of best fit for the model projection is listed in the last row of Table 1. The model projects a total of 8251 trichoid sensilla for subsegments 3–75 and 8509 for subsegments 3–80. The projected sum from the regression of actual counts from 180 to 200 mg insects, listed in the fourth row of Table 1, was 8431 sensilla for subsegments 3–75 and 8614 for subsegments 3–80, a difference of only 2%. This is to be compared with the total of 9241 sensilla estimated by Jefferson *et al.* (1970), a difference of 8%. For males, the predicted numbers of Type I, II and III sensilla were 121.6, 25.0 and 13.0 respectively, compared to average actual counts of 11800, 28.3 and 13.5 respectively. The only differences we observed between the sexes was in the proportions of the three types of sensilla trichodea.

An accurate accounting of either the total number and/or type of sensilla trichodea in the model is difficult because the location of the gain or loss of surface area may not be proportional among all subsegments. Also, we did not measure the effect of a change in the number of subsegments relative to weight change, and we assumed, without any supporting evidence, that the increased antennal surface area of insects over 200 mg and the decreased surface area of animals under 200 mg is shared proportionally over the entire length of the antenna.

In comparing pupae of different weights, the model predicts that distributing a minimal increase of 0.007 mm^2 surface area, per mg increase in weight, over an antenna of 75 subsegments would result in an average increase of $93 \mu\text{m}^2$ surface area per subsegment. Disregarding the negligible differences at the basal region of the antenna both in the

fraction (Fig. 9) of occupied surface area and in sensillar density (Fig. 6), a simple proportion provides a calculated 0.25 additional sensilla per subsegment/mg of body weight. The difference, distributed over 75 subsegments, amounts to 19 additional sensilla per antenna/mg pupal weight. Thus, from the range of pupal weights actually obtained we can project that adult antennae from pupae weighing 150 mg project $8251 - 950 = 7301$ sensilla; and adults from pupae weighing 300 mg project a total of $8251 + 1890 = 10,141$ sensilla.

For adults the antennal surface increases $0.008 \text{ mm}^2/\text{mg}$ or about $107 \text{ }\mu\text{m}^2$ additional surface area/subsegment. When the same assumptions of evenly distributed surface area, occupied surface area, and sensillar density discussed previously were used in this calculation, we found an average of 22 additional sensilla per antenna/mg adult weight.

Applications to olfactory transduction

For studies of olfactory transduction, it may be necessary to make detailed investigations of morphological parameters of the antenna that have not been previously studied. For example, it may be important to know the number or surface area of the pores (within statistical limitations) on a particular type of sensillum, the surface area of a sensillum, or the distribution of a particular sensillum type on the antenna. In fact, we were able to distinguish differences in all three types of sensillum trichodea described by Jefferson *et al.* (1970) and to calculate density and numbers. Also, we were able to relate the number of sensilla to the surface area of the antenna, which, in turn, is related to the weight of the pupa or adult, information that we believe will be important to future morphological studies of antennae. The functional importance of sensilla trichodea is difficult to assess from a purely morphological viewpoint. The measurements in Table 2 do not show great differences between the antennae of males and females nor great differences in the number of sensilla trichodea which might imply some differential olfactory functionality. Simple calculations shows that the surface area of type I sensilla comprises 75% of the total olfactory sensillar surface area of the antenna of males and females, that of Type II sensilla comprises 14% (male) and 20% (female), and that of Type III sensilla comprises about 4 – 10% of the total sensillar surface area. The pegs of the sensillum coeloconica have only about 0.5% and the sensilla auricillica have only 1% of the sensillar surface area.

Naturally, we were greatly interested in identifying the type of sensillum innervated by cells which respond to (*Z*)-7-dodecen-1-ol acetate, the pheromone component emitted in greatest quantity from the pheromone gland surface. We expected to obtain some definitive morphological clues and to use a combination of electrophysiology and the SEM, as Albert *et al.* (1974) did, to identify these sensilla precisely. We were unable to obtain definitive data with the electrophysiology-SEM combination, but electrophysiological recordings of cells responding to (*Z*)-7-dodecen-1-ol acetate were obtained from sensilla that were clearly longer than Type III sensilla (O'Connell, unpublished; Mayer, unpublished). The pore density of Type II and III sensilla trichodea is closer to the density of the olfactory peg of the face fly, *Musca autumnalis* De Geer, (approx. $35 \text{ pores}/\mu\text{m}^2$) (Bay and Pitts, 1976), and the basiconic peg of the beetle, *Nebria brevicollis* (F.) (approx. $37 \text{ pores}/\mu\text{m}^2$) (Daly and Ryan, 1979) than to the density of the pheromone-sensitive sensillum of *B. mori* and *Antheraea pernyi* (Guerin) approx. $3 - 8 \text{ pores } \mu\text{m}^2$) (Schneider and Steinbrecht, 1968; Kaissling, 1971; Steinbrecht, 1973).

We therefore hypothesize that the Type I sensilla trichodea are innervated by the pheromone-sensitive primary olfactory receptor cells in the cabbage looper because: (a) the pore density of Type II sensilla more closely resembles that of sensilla which are associated with responses to a broad range of non-pheromone chemicals; (b) the external structure of Type I sensilla is similar to the external structure of the sensillum with the same functionality in *B. mori* (Steinbrecht, 1973); and (c) the pores of Type I sensilla trichodea were roughly of the same size and density as reported for pores of Type I sensilla trichodea from *B. mori*.

CONCLUSIONS

In this report we have attached importance to the relationship between flagellar surface area and the number of sensilla. This relationship will have to be considered in future morphometric evaluations of the antenna of other noctuids. No sufficiently detailed morphometric examinations of other noctuid antennae yet have been attempted, so the universality of the observation cannot be determined. However, it is conceivable that a survey of some of the animals that have been studied in great detail would be instructive. For example, there are two tortricid species for which some morphometric details are known, *Choristoneura fumiferana* (Clemens) (Albert and Seabrook, 1973) and *Cydia nigricana* Stephens (Wall, 1978). Also, some morphometric details are known of the antenna of *Manduca sexta* (L.) (Sanes and Hildebrand, 1976). In addition, the antenna of *B. mori* was studied extensively (Schneider and Kaissling, 1956, 1957, 1959; Steinbrecht, 1970), and if surface area affected the numbers of sensilla in this animal, it would doubtlessly have been observed. Other Lepidoptera whose antennae have been studied in detail are the saturniids, *Lymantria dispar* (L.) (Scheffler, 1975), *Antheraea* (= *Telea*) *polyphemus* (Cramer), *Antheraea pernyi*, *Hyalophora* (= *Platysamia*) *cecropia* (L.) and *Samia* (= *Philosamia*) *cynthia* (Drury) Boeckh *et al.*, 1960).

We hesitate to ascribe functional or evolutionary significance to these sensillar distributions, but it is interesting from the point of view of developmental biology to ponder briefly the constraints on sensillar development. Because of the constant density, the cells that develop into sensilla trichodea may be affected by spatial and chemical exigencies such as hypothesised by Schafer (1973), Lawrence (1970) and Wigglesworth (1940).

REFERENCES

- ADAM, G. and M. DELBRÜCK. 1968. Reduction of dimensionality in biological diffusion processes, pp. 198–215. In A. Rich and N. Davidson (eds.) *Structural Chemistry and Molecular Biology: A Volume Dedicated to Linus Pauling by His Students, Colleagues, and Friends*. W. H. Freeman, San Francisco.
- ALBERT, P. J. and W. D. SEABROOK. 1973. Morphology and histology of the antenna of the male eastern spruce budworm, *Choristoneura fumiferana* (Clem.). *Can. J. Zool.* **51**: 443–48.
- ALBERT, P. J., W. D. SEABROOK and U. PAIM. 1974. Isolation of a sex pheromone receptor in males of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.). *J. Comp. Physiol.* **91**: 79–89.
- ALTNER, H. 1977. Insect sensillum specificity and structure: An approach to a new typology, pp. 295–303. In J. LeMagnen and P. MacLeod, (eds.) *Olfaction and Taste VI*. Information and Retrieval, London.
- BAY, D. E. and C. W. PITTS. 1976. Antennal olfactory sensilla of the face fly, *Musca autumnalis* De Geer. *Int. J. Insect Morphol. Embryol.* **5**: 1–16.
- BOECKH, J., K.-E. KAISLING and D. SCHNEIDER. 1960. Sensillen und Bau der Antennengeißel von *Telea polyphemus* (Vergleiche mit weiteren Saturniden: *Antheraea*, *Platysamia* und *Philosamia*). *Zool. Jahrb., Abt. Anat. Ontog.* **78**: 560–84.
- BOECKH, J., K.-E. KAISLING and D. SCHNEIDER. 1965. Insect olfactory receptors, pp. 263–80. In L. Frisch, (ed.) *Cold Spring Harbor Symposia on Quantitative Biology Vol. 30, Sensory Receptors*. Cold Spring Harbor Laboratory, New York.

- BÖHM, L. K. 1911. Die antennalen Sinnesorgane der Lepidopteren. *Arb. Zool. Inst. Wien* **19**: 219 – 46.
- CALLAHAN, P. S. 1975. Insect antennae with special reference to the mechanism of scent detection and the evolution of the sensilla. *Int. J. Insect Morphol. Embryol.* **4**: 381 – 430.
- DALY, P. J. and M. F. RYAN. 1979. Ultrastructure of antennal sensilla of *Nebria brevicollis* (Fab.). *Int. J. Insect Morphol. Physiol.* **8**: 169 – 81.
- JEFFERSON, R. N., R. E. RUBIN, S. U. MCFARLAND and H. H. SHOREY. 1970. Sex pheromones of noctuid moths. XXII. The external morphology of the antennae of *Trichoplusia ni*, *Heliothis zea*, *Prodenia ornithogalli*, and *Spodoptera exigua*. *Ann. Entomol. Soc. Amer.* **63**: 1227 – 38.
- KAISSLING, K.-E. and E. PRIESNER. 1970. Die Riechschwelle des Seidenspinners. *Naturwissenschaften* **57**: 23 – 8.
- KAISSLING, K.-E. 1971. Insect olfaction, pp. 350 – 431. In L. M. Beidler (ed.) *Handbook of Sensory Physiology, Chemical Senses I, Olfaction*. Springer-Verlag, New York.
- LAWRENCE, P. A. 1970. Polarity and patterns in the postembryonic development of insects. *Adv. Insect Physiol.* **7**: 197 – 266.
- LIN, S.-H. and Y. S. CHOW. 1972. Sense organs of the antenna of the cabbage looper, *Trichoplusia ni*. *Ann. Entomol. Soc. Amer.* **65**: 296 – 99.
- SANES, J. R. and J. G. HILDEBRAND. 1976. Structure and development of antennae in a moth *Manduca sexta*. *Dev. Biol.* **51**: 282 – 99.
- SCHAFFER, R. 1973. Postembryonic development in the antenna of the cockroach, *Leucophaea maderae*: growth, regeneration and the development of the adult pattern of sense organs. *J. Exp. Zool.* **183**: 353 – 364.
- SCHAEFFLER, H. J. 1975. Der Bau der Antennen bei WZ- und ZZ-intersexen des Schwammspinners *Lymantria dispar* L. *Z. Morphol. Tiere* **80**: 203 – 27.
- SCHNEIDER, D. 1964. Insect antennae. *Annu. Rev. Entomol.* **9**: 103–22.
- SCHNEIDER, D. and K.-E. KAISSLING. 1956. Der Bau der Antenne des Seidenspinners *Bombyx mori* L. I. Architektur und Bewegungsapparat der Antenne sowie Struktur der Cuticula. *Zool. Jahrb. Abt. Anat. Ontog.* **75**: 287 – 310.
- SCHNEIDER, D. and K.-E. KAISSLING. 1957. Der Bau der Antenne des Seidenspinners *Bombyx mori* L. II. Sensillen, cuticuläre Bildungen und innere Bau. *Zool. Jahrb. Abt. Anat. Ontog.* **76**: 223 – 50.
- SCHNEIDER, D. and K.-E. KAISSLING. 1959. Der Bau der Antenne des Seidenspinners *Bombyx mori* L. III. Das Bindegewebe und das Blutgefäß. *Zool. Jahrb. Abt. Anat. Ontog.* **71**: 111 – 32.
- SCHNEIDER, D. and R. A. STEINBRECHT. 1968. Checklist of insect olfactory sensilla. *Symp. Zool. Soc. Lond.* **23**: 279 – 97.
- SCHOONHOVEN, L. M. and V. G. DETHIER. 1966. Sensory aspects of host-plant discrimination by lepidopterous larvae. *Arch. Néerl. Zool.* **16**: 499 – 503.
- STEINBRECHT, R. A. 1970. Zur Morphometrie der Antenne des Seidenspinners, *Bombyx mori* L.: Zahl und Verteilung der Riechsensillen. *Z. Morphol. Tiere* **68**: 93 – 126.
- STEINBRECHT, R. A. 1973. Der Feinbau olfaktorischer Sensillen des Seidenspinners. *Z. Zellforsch.* **139**: 533 – 65.
- WALL, C. 1978. Morphology and histology of the antenna of *Cydia nigricana* (F.). *Int. J. Insect Morphol. Embryol.* **7**: 237 – 50.
- WIGGLESWORTH, V. B. 1940. Local and general factors in the development of 'pattern' in *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* **17**: 180 – 200.
- ZACHARUK, R. Y. 1980. Ultrastructure and function of insect chemosensilla. *Annu. Rev. Entomol.* **25**: 27 – 47.