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The tympanal hearing organ of the parasitoid fly *Ormia ochracea* (Diptera, Tachinidae, Ormiini)

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Abstract. Tympanate hearing has evolved in at least 6 different orders of insects, but had not been reported until recently in the Diptera. This study presents a newly discovered tympanal hearing organ, in the parasitoid tachinid fly, *Ormia ochracea*. The hearing organ is described in terms of external and internal morphology, cellular organization of the sensory organ and preliminary neuroanatomy of the primary auditory afferents. The ear is located on the frontal face of the prothorax, directly behind the head capsule. Conspicuously visible are a pair of thin cuticular membranes specialized for audition, the prosternal tympanal membranes. Directly attached to these membranes, within the enlarged prosternal chamber, are a pair of auditory sensory organs, the bulbae acusticae. These sensory organs are unique among all auditory organs known so far because both are contained within an unpartitioned acoustic chamber. The prosternal chamber is connected to the outside by a pair of tracheae. The cellular anatomy of the fly's scolopophorous organ was investigated by light and electron microscopy. The bulba acustica is a typical chordotonal organ and it contains approximately 70 receptor cells. It is similar to other insect sensory organs associated with tympanal ears.The similarity of the cellular organization and tympanal morphology of the ormiine ear to the ears of other tympanate insects suggests that there are potent constraints in the design features of tympanal hearing organs, which must function to detect high frequency auditory signals over long distances. Each sensory organ is innervated by a branch of the frontal nerve of the fused thoracic ganglia. The primary auditory afferents project to each of the pro-, meso-, and metathoracic neuropils. The fly's hearing organ is sexually dimorphic, whereby the tympanal membranes are larger in females and the spiracles larger in males. The dimorphism presumably reflects differences in the acoustic behavior in the two sexes.

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Key words: Insect hearing – Acoustic parasitoid – Tympanal organ – Scolopophorous mechanoreceptor – Sexual dimorphism – Convergent evolution – *Ormia ochracea* (Insecta, Diptera)

Introduction

In insects, the ability to hear high frequency signals, greater than 2 kHz, over distances greater than a few meters requires the possession of a tympanal hearing organ. This is true of all of the so-called "auditory insects," which include members of the Orthoptera, Lepidoptera, Coleoptera, Neuroptera, Dictyoptera, and Hemiptera. Although the particular anatomical position and gross morphology of each hearing organ may vary between and even within orders, all tympanal hearing organs share a basic organizational plan (Michelsen and Nocke 1974; Michelsen and Larsen 1985). In this paper, we describe the anatomy of a tympanal hearing organ in the order Diptera, in parasitoid flies of the genus *Ormia* (Family Tachinidae, Tribe Ormiini). Our description follows up the recent discovery of tympanate hearing in dipterans (Lakes-Harlan and Heller 1992; Robert et al. 1992).

Tympanal hearing organs are characterized by a diagnostic cluster of morphological characters: *externally*, the presence, of a well-differentiated cuticular tympanal membrane, upon which the minute pressure changes of a transmitted sound stimulus play and, *internally*, the appositional association of the tympanal membrane with one or more air-filled sacs (tracheae) or chambers, and the attachment of a chordotonal, or scolopophorous, sensory organ to the air-filled sacs, or air-chambers, or to the tympanal membrane itself (Dethier 1963; Michelsen and Larsen 1985; Yack and Fullard 1990). Hearing organs generally occur in bilaterally symmetric pairs, one on each side of the mid-sagittal plane of the body. In fact, in all auditory vertebrates and insects, the anatomical separation of the ears is a key element for the processing

of directional sound cues. In this ormiine fly, the lack of anatomical separation in the hearing apparatus raises the question of how this insect can detect the location of a pinpoint sound source.

Some dipterans are well-known for their ability to produce and detect acoustic signals. Mosquitoes and many species of the genus *Drosophila* use so-called "love songs" as part of their courtship and mating behavior (mosquitoes: Johnston 1855; Mayer 1874; drosophilid flies: Bennet-Clark and Ewing 1967; review: Roth 1948). The frequencies contained in these reproductive signals are relatively low, ranging from 100 to 600 Hz (Mayer 1874; Bennet-Clark and Ewing 1968). Mate attraction and courtship behavior take place at close quarters, ranging from one or two body lengths in *Drosophila* to a few decimeters in mosquitoes (Markl 1973).

The fly *O. ochracea* is an acoustic parasitoid. The larviparous female flies locate their host species (field crickets such as *Gryllus rubens* or *G. integer*) by hearing and homing in on the male cricket's calling song, which the cricket produces to attract potential mates (Cade 1975; Mangold 1978; Walker 1986). Gravid female flies must be able to detect the 5-kHz calling songs of their cricket hosts over distances of possibly many tens of meters. The female fly locates a singing male cricket and deposits a few maggots (first instar larvae) on or around him. The minute (400-μm-long) first instar maggots burrow into the host's body, where they feed and grow for about a week, after which they burrow out of the still-living host, and form pupana outside. The host dies shortly after the egress of the mature maggots. The adult fly emerges from the puparium after another week to 10 days. Adult flies are free-living and, considering the anatomy of their mouth parts, are presumably nectar or other fluid feeders. Mating occurs within 10 days, but nothing is known about mating behavior, except that it does not involve loud, cricket-like acoustic signals. This may be a factor in the sexual dimorphism in hearing organs, which is also reported in this paper. After another 10 days or so, female flies, gravid with larvae, fly out at dusk in their acoustically-guided search for cricket hosts. Thus, to complete reproduction, female flies must find crickets in which their maggots develop. To reproduce, a female ormiine fly must accomplish the same acoustic task as a female cricket: detect and locate a singing male cricket over long distances (at least 10 m). This strong similarity of selective pressures has resulted, by convergent evolution, in the case of this ormiine fly, in the evolution of a tympanal ear, the first of its kind to be described in the Diptera.

Presumably, tympanal hearing places certain constraints on the morphological design features, constraints that are dictated by the physical acoustics of sound transmission and detection (Michelsen and Nocke 1974). In effect, if a fly is to perform the same auditory detection task as a cricket, a katydid, a cicada, or a moth, it should possess a cricket- or mothlike ear. Presumably, this came about by convergent evolution, as has been suggested in other auditory insects (Yack and Fullard 1990). While there may appear to be a wide diversity in the kinds of tympanal ears among auditory insects, in fact all of these hearing organs show convergence of form and function. The existence of similar characteristics in all known tympanal hearing organs suggests that developmental mechanisms, as well as the laws of physical acoustics, set constraints in the building of an ear sensitive to high frequencies that can operate over long distances.

The task of locating a singing cricket host is accomplished exclusively by female *O. ochracea*. However, males also possess a prosternal hearing organ. This paper describes the morphology of the ormiine tympanal organ, presents a case of sexual dimorphism, discusses morphological similarities and differences with respect to other tympanal ears, and relates these findings to how this ear accomplishes the task of detecting cricket songs.

Materials and methods

Animals

The parasitoid fly *Ormia ochracea* was used in this study. Wild flies were captured in the field near Gainesville, Fla., or at the Gulf Coast Research and Education Center at Bradenton, Fla. (for method of capture, see Walker 1986). The flies used for most of the laboratory work were first to fifth generation adult flies from the laboratory colony, reared from the wild-caught flies. The methods of culture and rearing of ormiine flies were those of Wineriter and Walker (1990). Briefly, the flies were housed in cylindrical polycarbonate containers (diameter: 29.5 cm, height: 45 or 60 cm) where they had free access to liquid hummingbird diet as a food source. The average temperature in the rearing room was 25.5°C (SD: ± 1°C, $n=21$), and the relative humidity was 55% (SD: ± 3%, $n=21$). The light regime was a 16:8 h light: dark period. The fly colony was maintained as a crowded population with about 90 flies per container.

Terminology

The term *hearing organ* refers here to the entire anatomical apparatus used for air-borne sound reception by the fly. The hearing organ comprises the modified inflated prosternum, the tympanal membranes and associated internal membranes, the connected tracheal system, and the sensory organs containing the scolopidial mechanoelectric transducers. By analogy to the cricket's *crista acustica* (Michel 1974), the sensory organ of *O. ochracea* was named the *bulba acustica*, for its bulbous shape and auditory function.

Dissections

The gross internal anatomical features of the hearing organ were investigated by dissecting fresh animals or animals preserved in alcoholic Bouin's. Occasionally, methylene blue stain was used in fresh dissections to enhance contrast between cuticle, muscles, nervous system and tracheae. Camera lucida line drawings were made from such dissections to depict the relative positions of the various parts of the hearing organ.

Morphometrics

To compare the anatomy of male and female hearing organs, measurements of the tympanal region and the mesothoracic spiracles were made on live, air-dried and alcohol-preserved specimens, using a calibrated eyepiece micrometer.

The width of the prosternum and the thorax was measured for 16 females and 7 males. The surface area of the prosternal tympanal

membranes was measured as follows. The area of the prosternal inflation that was identified as being thin, membranous, semitransparent cuticle was outlined on a scanning electron micrograph (for 5 females and 5 males). The surface area was determined by cutting out the outlined surfaces, weighing them and calibrating them against a reference surface area of known weight. The length and width of the spiracles were also measured (for 16 females and 7 males) to determine the surface area of the opening of the mesothoracic spiracles. The surface area of the opening was calculated as the surface area of the ellipse fitted around the length and width of the spiracle. The number of sensory cells contained in the bulba acustica was determined by directly counting the scolopale cells from serial histological sections along longitudinal and transverse planes. Counts were made from 18 cm × 24 cm photographic prints of the histological sections (for 3 females and 2 males). Typically, in scolopophorous organs, each scolopale cell is associated with a single sensory cell (Gray 1960; Michel 1974). The scolopale cells were morphologically identifiable by their scolopale caps or the characteristic arrangement of the scolopale rods. Statistical comparisons were made using a Mann-Whitney U-test at a rejection level of 0.05 (Zar 1984).

Histology

The intact thoraces of 31 flies were preserved in alcoholic Bouin's fixative, embedded in paraffin wax or JB4 plastic (Polysciences Inc.), and sectioned horizontally, transversely, or sagittally, to reconstruct the internal geometry of the hearing organ. Plastic sections (JB4, 5 μm) of the entire thorax and associated cuticular structures were stained with 0.5% toluidine blue in 0.5% borax. Masson trichrome stain (Baker modification; Pantin 1946) was used on paraffin sections (8 μm)for differentiating the cuticle from the nervous system as well as for specifically staining scolopale rods and caps.

Cobalt backfills

The central nervous projections of the peripheral axons from the sensory organ were revealed by means of the cobalt backfill technique. Hand-pulled micropipettes were filled with 4% cobalt chloride and inserted into the bulba acustica after partial removal of one of the tympanal membranes. In general, at least 3 h (at room temperature) were required for the cobalt solution to diffuse through or along the sensory axons back to their terminals in the thoracic ganglia. After precipitation of the cobalt with hydrogen sulfide, the ganglion was fixed in Carnoy's or Bouin's fixative for 1 h. The ganglion was dehydrated in ethanol (95%, 100%, 100%, for 10 min each) and cleared in methyl salicylate. Ganglia were first examined in methyl salicylate and subsequently embedded in paraffin, sectioned at 10 μm, and intensified (Timm's technique) to reveal the afferent projections in the thoracic neuropils. The data are based on 19 successfully stained ganglia.

Scanning electron microscopy (SEM)

The surface structures of prosternal tympanal membranes and tympanal pits were examined by scanning electron microscopy. After removal of the heads, the thoraces of both female and male flies were air-dried and sputter-coated with gold. Specimens were then examined and photographed on an AMR-1000A scanning electron microscope.

Transmission electron microscopy (TEM)

The anterior portion of the prothorax was dissected and fixed for 3 h in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), followed by a postfix treatment of 1 h in 2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4). The preparations then were dehydrated in an alcohol series (70%, 95%, 3 × 100%, for 10 min each) and cleared in propylene oxide for 2 × 15 min. After embedding in Polybed (Polysciences Inc.), the preparations were sectioned at 90 nm with a Reichert-Jung Ultracut E ultramicrotome. Sections were stained first with saturated aqueous uranyl acetate for 10 min and then with 4% lead acetate for 5 min (Venable and Coggeshall 1965). Examination and photography of selected sections took place on a Phillips EM201 transmission electron microscope.

Results

The hearing organ: morphological overview

The hearing organ of *O. ochracea* is located on and within the prothorax, but is normally hidden from view behind the insect's large head (Fig. 1). It is located directly behind the posterior and ventral edge of the head capsule and anterior to the prothoracic pair of coxae. Viewed externally, the hearing organ is a bladderlike inflation of the median prosternal sclerite, the prosternum. The inflated prosternum extends medially from the base of the prothoracic coxae to the cervical sclerites of the neck (Fig. 1A, B). This system of sclerites provides a rigid cuticular support for a pair of thin, supple membranes, the prosternal tympanal membranes (*PTM*s). These membranes form the anterior face of the single, air-filled prosternal chamber. Fig. 1B shows a close-up sagittal view of the hearing organ, after the lateral cuticular structures have been removed by dissection to allow visualization of the organ's internal anatomy.

The prosternal chamber contains a pair of bulbous, cellular structures, each of which is an auditory sensory organ, the bulba acustica (*BAc*). The shape of the *BAc* is approximately ellipsoid, about 170 μm long and 45 to 55 μm wide. Each *BAc* is attached by its anterior end to a stiff rod – the auditory apodeme (*AA*) – that projects longitudinally into the prosternal chamber (Fig. 1B). Each bilaterally symmetric *AA* inserts directly upon a *PTM*, on a patch of thickened cuticle, the tympanal pit (Fig. 2). Each bulba acustica is also connected to the posterior wall of the prosternal chamber by a cordlike suspensory ligament (*SL*). Finally, the *BAc*, the *AA* and the *SL* are attached to the roof of the prosternal chamber by a veil-like, continuous thin membrane, the internal accessory membrane (Fig. 1B). The prosternal chamber opens to the outside through a bilateral pair of tracheae, each of which opens out through a mesothoracic spiracle (Fig. 1B).

In frontal view, the external morphology of the hearing organ is bilaterally symmetrical (Figs. 2, 3). The triangular prosternal inflation of thick cuticle protrudes from between the prothoracic coxae to meet the prothoracic base of the neck, the cervix. On either side of the dorsoventral line of symmetry is the pair of forward-facing prosternal tympanal membranes (*PTM*s). In the female, each fan-shaped *PTM* displays prominent radial corrugations, which converge upon a region of thicker cuticle, the tympanal pit, which is the point of internal attachment of the *PTM* to the auditory apodeme

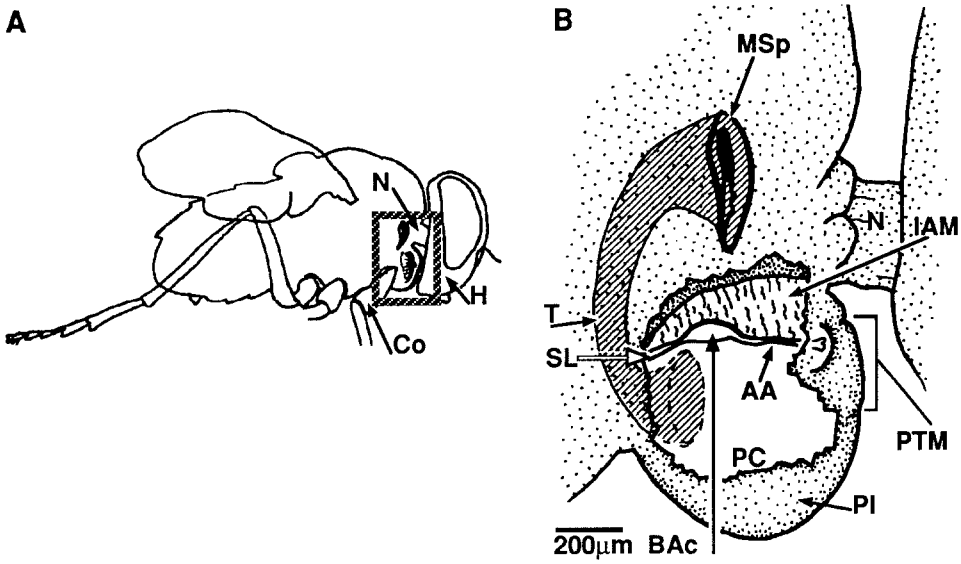


Fig. 1A,B. Anatomy of the hearing organ of *Ormia ochracea*. **A** Line drawing of the fly highlighting the location of the hearing organ (*hatched box*). The prosternal inflation forming the ear bulges out between the prothoracic coxae (*Co*) and the neck insertion (*N*). The hearing organ sits directly behind the head capsule (*H*). **B** Sagittal view of the hearing organ. The inflated prosternum (*PI*) forms the cuticular boundary of the single prosternal chamber (*PC*) where the sensory organs, the bulbae acusticae (*BAc*), are situated. From its anterior end, each *BAc* is connected to the prosternal tympanal membrane (*PTM*) by a stiff cuticular rod, the auditory apodeme (*AA*). On the other end, a suspensory ligament (*SL*) attaches the *BAc* to the posterior wall of the *PC*. The very thin internal accessory membrane (*IAM*) connects the *AA*, the *BAc*, and the *SL* to the roof of the prosternal chamber. The prosternal chamber is not a closed internal environment, it is in contact with the outside via a pair of tracheae (*T*) that lead to the mesothoracic spiracles (*MSp*). *Scale bar*: 200 µm

(Figs. 1B, 2A). Also shown in Fig. 2A, on each side of the thorax, are the mesothoracic spiracular openings that connect the prosternal chamber to the outside via tracheae.

The sexual dimorphism of the hearing organ

In *O. ochracea*, the female and male tympanal hearing organs are prominently sexually dimorphic (see size difference in Figs. 2 and 3). Their respective morphology differs with respect to the size and shape of both the tympanal membranes and the spiracular openings, as described below.

The tympanal membranes. The fan-shaped *PTM*s of the female fly are wide and span almost the whole width of the thorax, from the midline to the base of the proepisternal setae (Fig. 2A). In contrast, the male *PTM*s are much reduced and do not extend to or cover the proepisternum, nor do they extend to the base of the proepisternal setae (Fig. 2B). The prosternal inflation is larger in females and supports a larger prosternal chamber. The overall span of the female hearing organ is significantly larger; it is 1.68 ± 0.19 mm wide (mean ± SD, $n=16$), as opposed to 1.05 ± 0.04 mm ($n=7$) in the male (see Table 1). In terms of surface area, female *PTM*s are 4.4 times larger than those of males (Table 2), and these calculations do not even take into account the differences in body size between the sexes, in which males are consistently bigger (Table 1).

Like other insect tympana, the female *PTM* is composed of thin (1 µm) and transparent cuticle. In contrast, the male *PTM* is 2 to 3 µm thick and opaque. Moreover, the female *PTM* is thrown into numerous fanlike corrugations (Figs. 2A, 3A), which are absent from the male *PTM* (Figs. 2B, 3B). For both sexes, the cuticle surrounding the tympanal pit is thicker and more rigid than on the rest of the *PTM*.

Table 1. Morphometric measurements of the prosternal region of female and male *O. ochracea*

	Prosternal width [mm]	Thorax width [mm]	Ratio prosternum: thorax
Female ($n=16$)	1.68 ± 0.19	2.30 ± 0.22	0.73
Male ($n=7$)	1.05 ± 0.04	2.66 ± 0.15	0.39
U-test	$P<0.001$	$P<0.002$	

Prosternal width is defined as the length across the most distal parts of the prosternal tympanal membranes (PTMs); thorax width is the width of the mesothorax. Values are given as means with their standard deviations

Table 2. Surface area of the prosternal tympanal membrane (PTM) and of the mesothoracic spiracle (MSp) for female and male *O. ochracea*

	Surface of the PTM (mm^2 ± SD)	Surface of the MSp (mm^2 ± SD)	Ratio PTM:MSp
Female	0.288 ± 0.056 ($n=5$)	0.06 ± 0.01 ($n=16$)	4.8
Male	0.065 ± 0.009 ($n=5$)	0.12 ± 0.09 ($n=7$)	0.5
U-test	$P<0.01$	$P<0.001$	
Female/male	4.4	2.00	

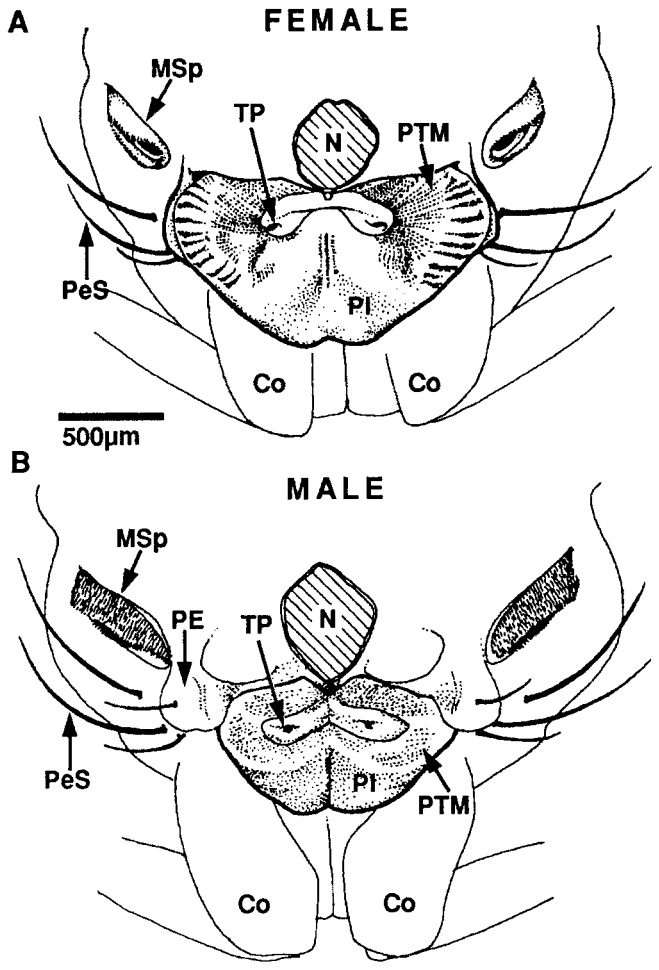


Fig. 2A,B. Line-drawings of the prosternal region illustrating the external anatomy of the hearing organ. In frontal view, the prosternal inflation (*PI*) sits between the prothoracic coxae (*Co*) and the neck insertion (cervix, *N*, *hatched area*). The two bilateral depressions are the tympanal pits (*TP*). These are thicker cuticular locations on the *PTM* upon which both auditory apodemes insert. The spiracular inputs (*MSp*) to the interior of the *PC* are situated dorsolaterally on the mesothorax. **A** In females, the prosternal tympanal membranes (*PTMs*) extend laterally to the base of the proepisternal setae (*PeS*). Also, the *PTMs* possess radial corrugations. **B** The male hearing organ also features a pair of prosternal tympanal membranes, that are much reduced in surface area, thicker, and lack the radial corrugations. The membranous area does not extend further laterally than the median ridge of the proepisternum (*PE*). The male mesothoracic spiracles (*MSps*) open into tracheae leading to the *PC*. In contrast to in females, in males the *MSps* present a larger surface area and are surrounded by a dense array of microtrichae. *Scale bar*: 500 µm

The mesothoracic spiracles. In both females and males, the prosternal chamber opens to the outside by a bilateral pair of tracheae connected to the mesothoracic spiracles (Figs. 1B, 4). The spiracular openings are conspicuously larger in males than in females, males presenting a spiracular surface area of 0.12 ± 0.09 mm^2 ($n=7$) but in females 0.06 ± 0.01 mm^2 ($n=16$; Table 2). By observing live, cold-anesthetized flies under the microscope, it was seen that both males and females can control the opening of the spiracles by retractile, supple spiracular valves (Fig. 4A). In the female the cuticular rim, the peritreme, bordering the entrance of the spiracle, is barren of hairs and features, anteriorly, a nonmobile cuticular flap that partially covers the opening (Fig. 4A). Besides being larger, the spiracles of males are anatomically distinguishable from those of the female by the numerous and contiguous feathery hairs, the microtrichae, situated around the peritreme (Fig. 4B).

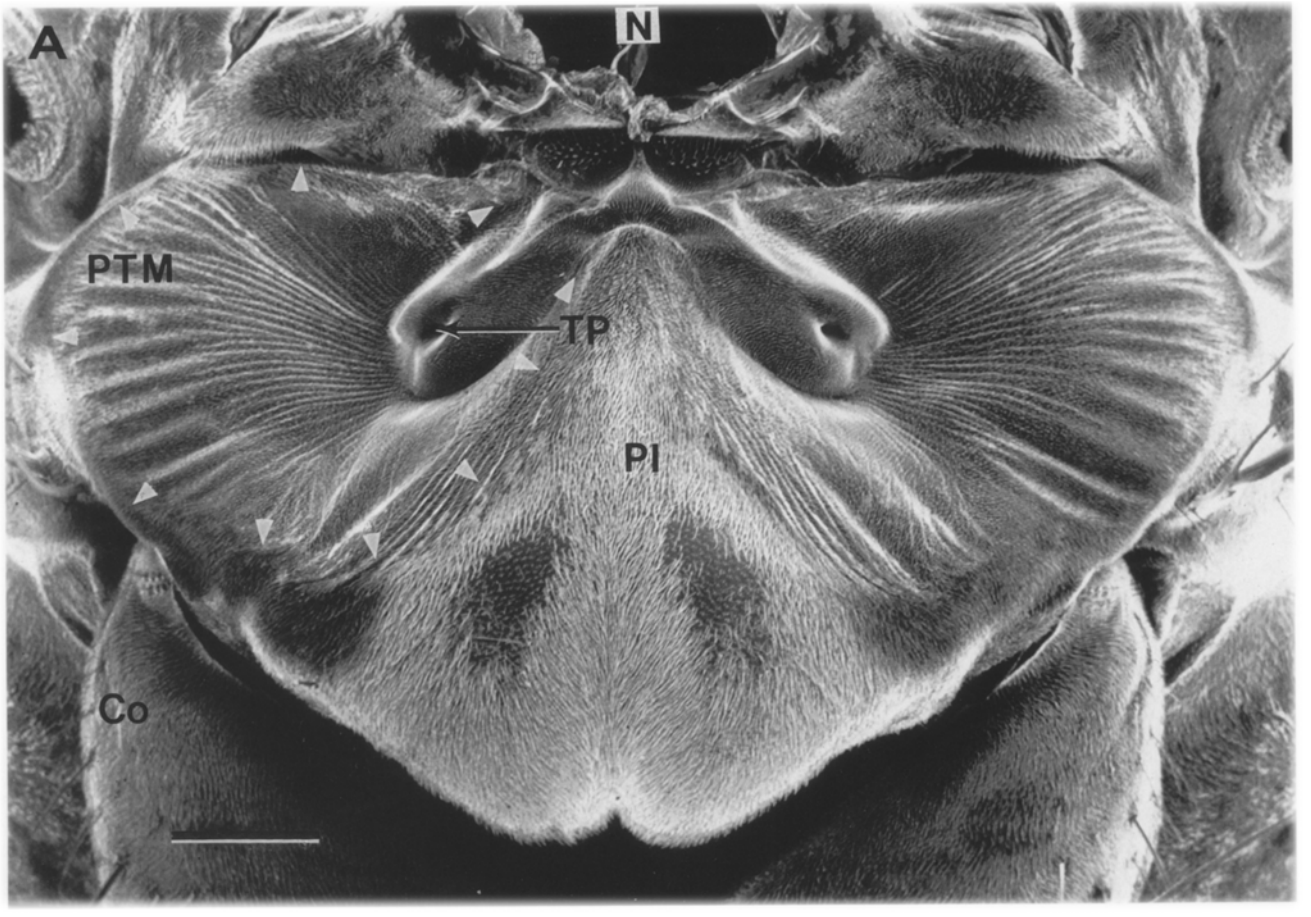
The prosternal chamber

The internal architecture of the hearing organ was revealed by examination of serial histological sections made along the longitudinal and horizontal axes of the fly. This investigation firmly established the fact that the two sensory organs (*BAc*) are situated within an undivided space formed by the prosternal chamber (Fig. 5). Unlike any other auditory organ, there is no median septum partitioning the prosternal chamber into two separate acoustic spaces. A thin internal accessory membrane (*IAM*) connects the *BAc* to the roof (dorsal boundary) of the prosternal chamber (Fig. 5). The *IAMs* are less than 1 µm thick, and are thinner than the *PTMs*.

A bilaterally symmetric pair of trachea connects the mesothoracic spiracle to the inside of the prosternal chamber. The internal openings of the tracheal tubes are closely adjacent to each ipsilateral *BAc* (Fig. 5).

The fused cervical connectives of the ventral nerve cord run directly atop the roof of the prosternal chamber (Fig. 5). They connect the fused thoracic ganglia (posterior to the depicted section) to the cerebral neural complex (brain). A horizontal longitudinal section of the fly's thorax is shown in Fig. 6A to depict the position of the prosternal chamber and the *PTM* with respect to the fused thoracic ganglia. Both *BAc* (sectioned longitudinally) are situated in a single space. This represents a unique feature of the hearing organ of *O. ochracea*. In this view, they have been slightly displaced during the embedding procedure; however, the location shown in Fig. 5 is typical of that in the intact animal. The two *PTMs* form the anterior boundary of the prosternal chamber. They are separated medially by a small segment of thicker cuticle. The thin internal accessory membranes are also visible, running from the *BAc* to the *PTM*. Because of its prominence in Fig. 6A, it is worth pointing out the large haltere nerve, which enters the thoracic ganglia posteriorly. This nerve contains at least 500 axons from the campaniform sensilla of the haltere gyroscopic organ (Sandeman and Markl 1980; Strausfeld and Seyan 1985). Note, also, the three thoracic neuromeres (Fig. 6 A).

Fig. 6B shows a schematic, composite diagram of the anterior thorax in a horizontal longitudinal section. Each *BAc* is mechanically coupled to the tympanal pit and the *PTM* by the auditory apodeme. The suspensory ligament attaches the *BAc* to the posterior wall of the prosternal chamber. Each *BAc* is adjacent to the internal opening of the trachea that in turn leads to the mesothoracic spiracle, and to the outside. Anatomical reconstructions based on serial sections indicate that these tracheal tubes have the shape of a double-ended horn (Fig. 6B). The arrow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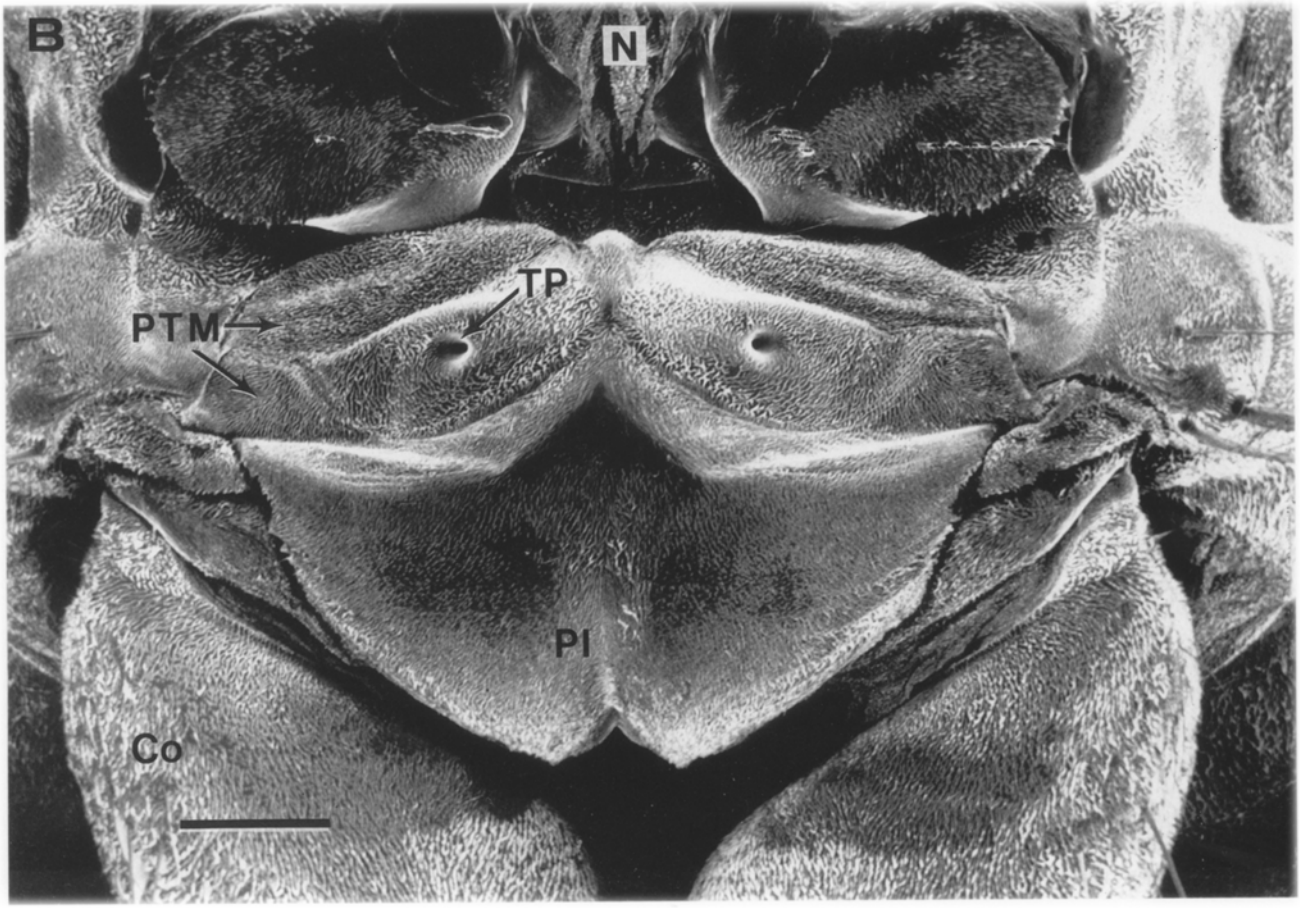
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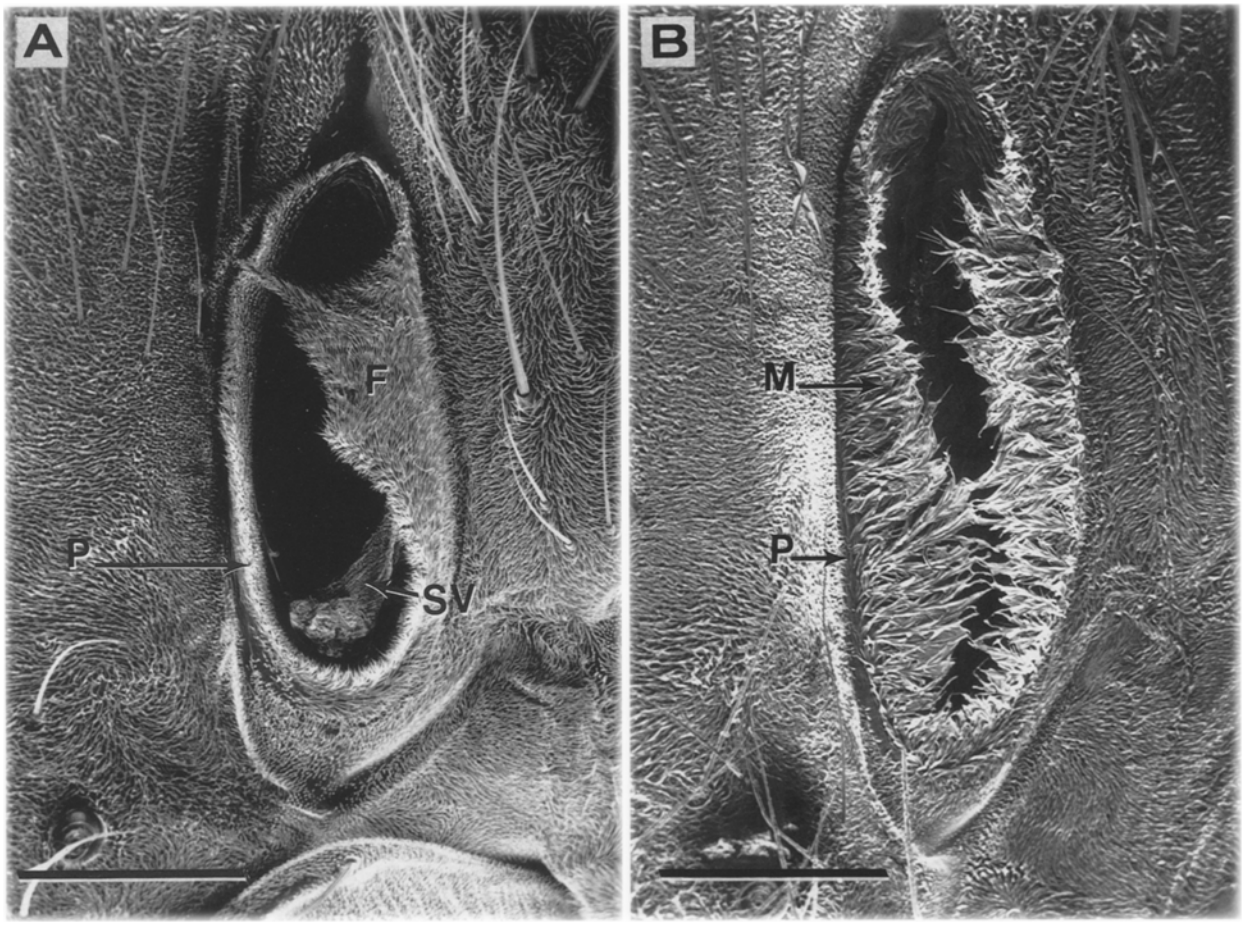


Fig. 4A,B. Scanning electron micrographs illustrating the sexual dimorphism of the mesothoracic spiracles. *Scale bars*: 200 µm, × 155. **A** Female spiracular opening, bordered by a naked peritreme (*P*), the anterior, thicker edge of which forms a flap (*F*) partially covering the opening. Ventrally, one of the two spiracular valves (*SV*) controlling the aperture of the spiracle can be seen in the open position. **B** Male spiracle featuring a peritreme (*P*) with feathery peritremal microtrichae (*M*). Situated behind the bilateral arrays of microtrichae, the retractable valves are not visible here

drawn in both BAc represents the scolopidia within the sensory organ. The bulba acustica is innervated by the auditory nerve from the prothoracic neuromere. The auditory nerve branches from the frontal nerve before entering the prosternal chamber.

The cellular anatomy of the sensory organ

The cellular anatomy of the bulba acustica is typical of insect chordotonal sensory organs involved in tympanal hearing (Gray 1960; Howse 1968; Michel 1974; Moulins 1976). The basic structural unit of the sensory organ is the scolopidium: a multicellular arrangement comprising a bipolar sensory neuron, several accessory cells, and a scolopale cell. The presence of such scolopidial arrangements in the bulba acustica is demonstrated by the light-microscopy micrographs of Fig. 7. The key features of a scolopidium, seen in cross section, are the hexagonal arrangement of the scolopale rods (sometimes also arranged in a heptagonal or octagonal symmetry), the sensory cilium of a bipolar sensory neuron and the scolopale cap (Fig. 7A). The serial cross sections ($n = 5$ animals) of the *BAc* provided views of scolopidia sectioned at different levels: from the scolopale cap to, progressively, the region of the proximal fusion of the scolopale rods, dendrites, and perikarya of the sensory neurons. In some of these cross sections, groups of scolopidia sectioned at roughly the same level could be distinguished (Fig. 7A, *black arrowheads*). In each longitudinal 5-µm-thick section, however, a regular dorsoventral alignment of the scolopidia is very distinct, along with the scolopale caps, the scolopale rods, the dendritic cilia, and the cell bodies of the sensory neurons (Fig. 7B). Anteriorly, a group of attachment cells mechanically couples the scolopidia to the tip of the *BAc*. The total number of scolopidia in the

Fig. 3. Scanning electron micrographs of the female (**A**) and male (**B**) hearing organ showing in detail the structures described in Fig. 2. Abbreviations: prothoracic coxa (*Co*); neck insertion on the prothorax (*N*); prosternal inflation (*PI*); prosternal tympanal membrane (*PTM*); tympanal pit (*TP*). Scale bars: 200 µm. Magnification: × 100. **A** Frontal view of the female hearing organ. The *arrowheads* show the border of the *PTM*. **B** Frontal view of the male hearing organ

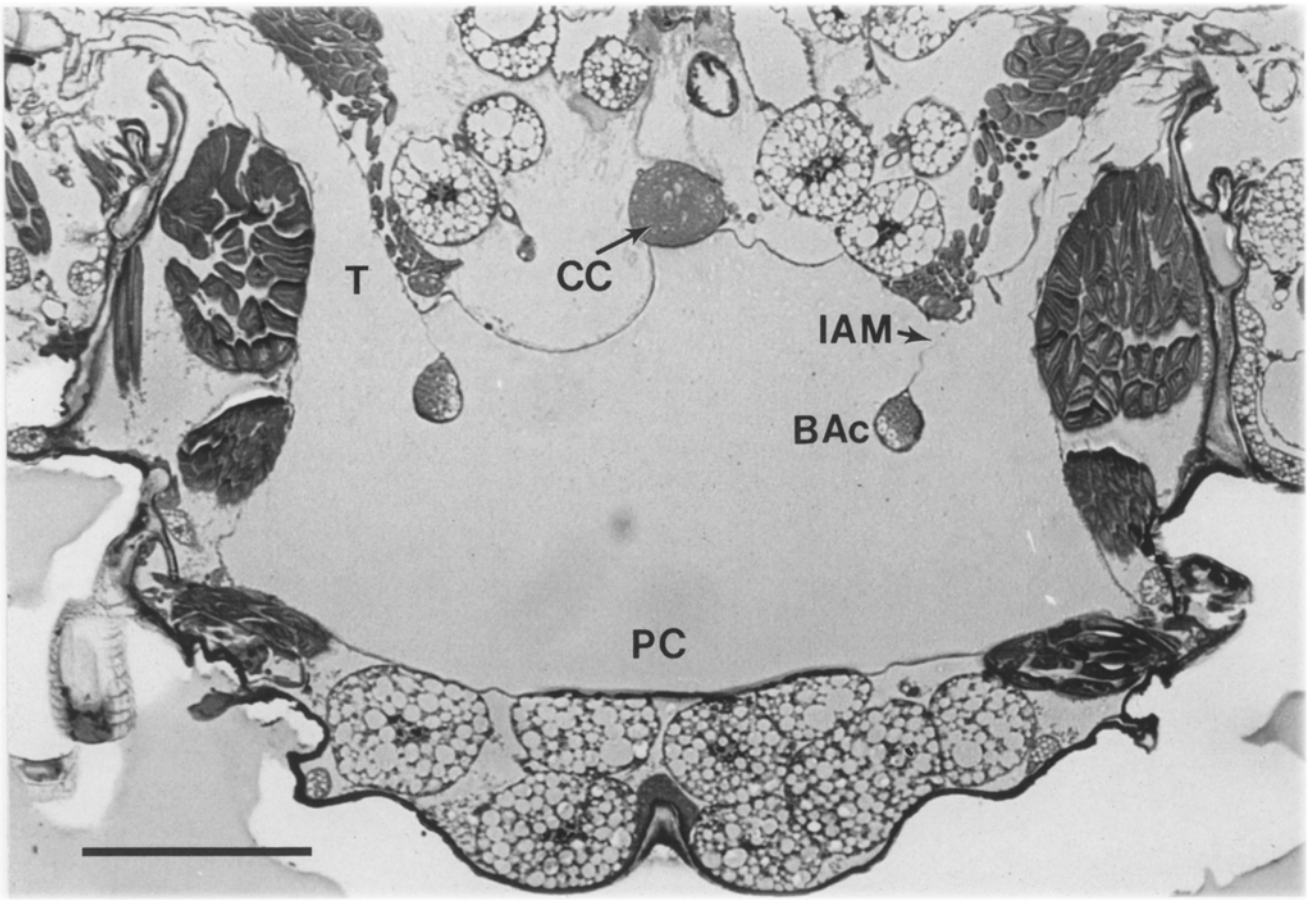


Fig. 5. Histological transverse section of the hearing organ; toluidine-blue staining. Directly facing the tracheae (*T*) from the mesothoracic spiracles, the two bulbae acusticae (*BAc*) are situated within the same undivided, acoustic space formed by the prosternal chamber (*PC*). The *BAc* is connected to the dorsal boundary of the (*PC*) by the internal accessory membrane (*IAM*). The cervical connective (*CC*) of the central nervous system can be seen medially, atop the *PC*. *Scale bar*: 100 μm; × 300

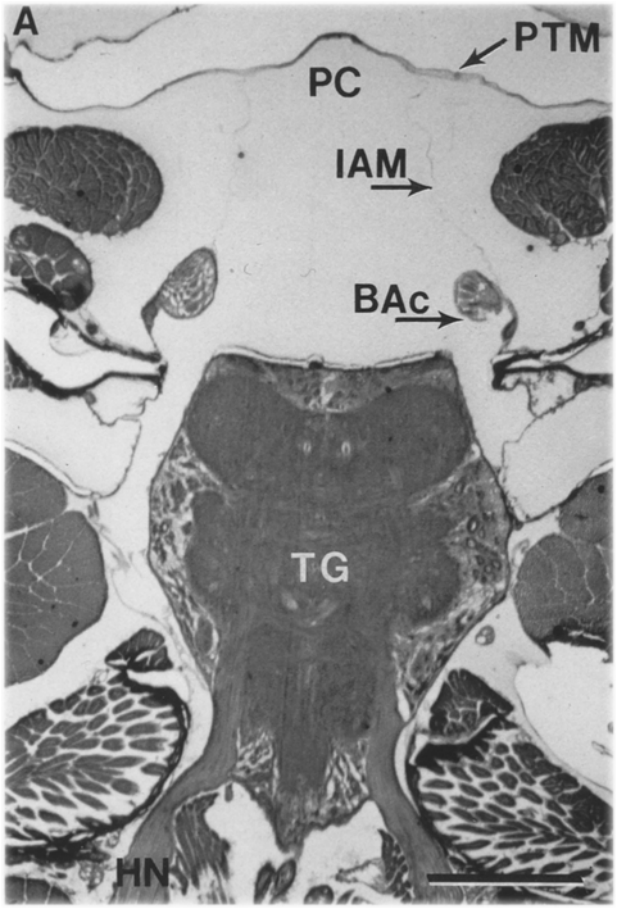
sensory organ was assessed by examining 18 cm × 24 cm prints of serial cross sections. For three female *O. ochracea*, the counts indicated the presence of 72, 65, and 74 scolopidia in the *BAc*. For two males, 75 and 76 scolopales were counted.

The scolopidium

In all acoustic insects, the scolopidia dedicated to hearing show common structural features (Gray 1960, reviews by Moulins 1976; Yack and Roots 1992). The characteristic structure of a scolopidium is that it is composed of three cells: a bipolar sensory neuron, with its axon, dendrite and cilium; a scolopale cell, which builds a sleeve around the dendrite and the cilium; and an attachment cell.

To investigate the possible common features between the dipteran tympanal ear and other insect ears or chordotonal organs, the ormiine scolopidium was reconstructed by studying high-magnification optical micrographs of longitudinal sections through scolopidia (like Fig. 7B) and especially transmission electron micrographs of (TEM) transverse sections (Fig. 9). According to the current schemes and terminology for classifying scolopidia (following Yack and Roots 1992; see also Young 1973; Moulins 1976), the present scolopophorous arrangement belongs to the types referred to as *mononematic*, one with a scolopale cap and a structurally associated 9 + 0 microtubular organization, and as *monodynal*, one with a single sensory cell per scolopale unit (Fig. 8).

Fig. 8 is a diagrammatic longitudinal reconstruction of a scolopidium. Noteworthy are the diagnostic scolopale cap and scolopale rods of the scolopale cell surrounding the cilium of the sensory neuron. At the proximal end of the scolopale rods is the dendritic collar, closely apposed to the dendritic apex of the sensory neuron. Half-way between the dendritic collar and the scolopale cap is an area where fine "spokes" of electron-dense material run from the cilium to the scolopale rods. These fine spokes can also be seen in Fig. 7B. This structure is reminiscent of the granular area described by Yack and Roots (1992) for the scolopidia of a lepidopteran chordotonal organ. Between the spokes and the scolopale cap is the ciliary dilation, which is similar to that described for other insect scolopidia (for recent review about this specific aspect, see Yack and Roots 1992). On the proximal side of the scolopidial complex are two accessory



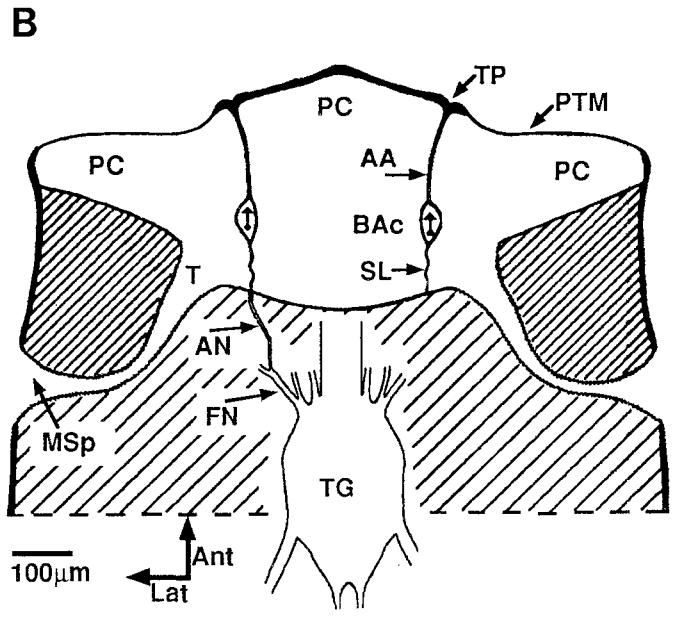


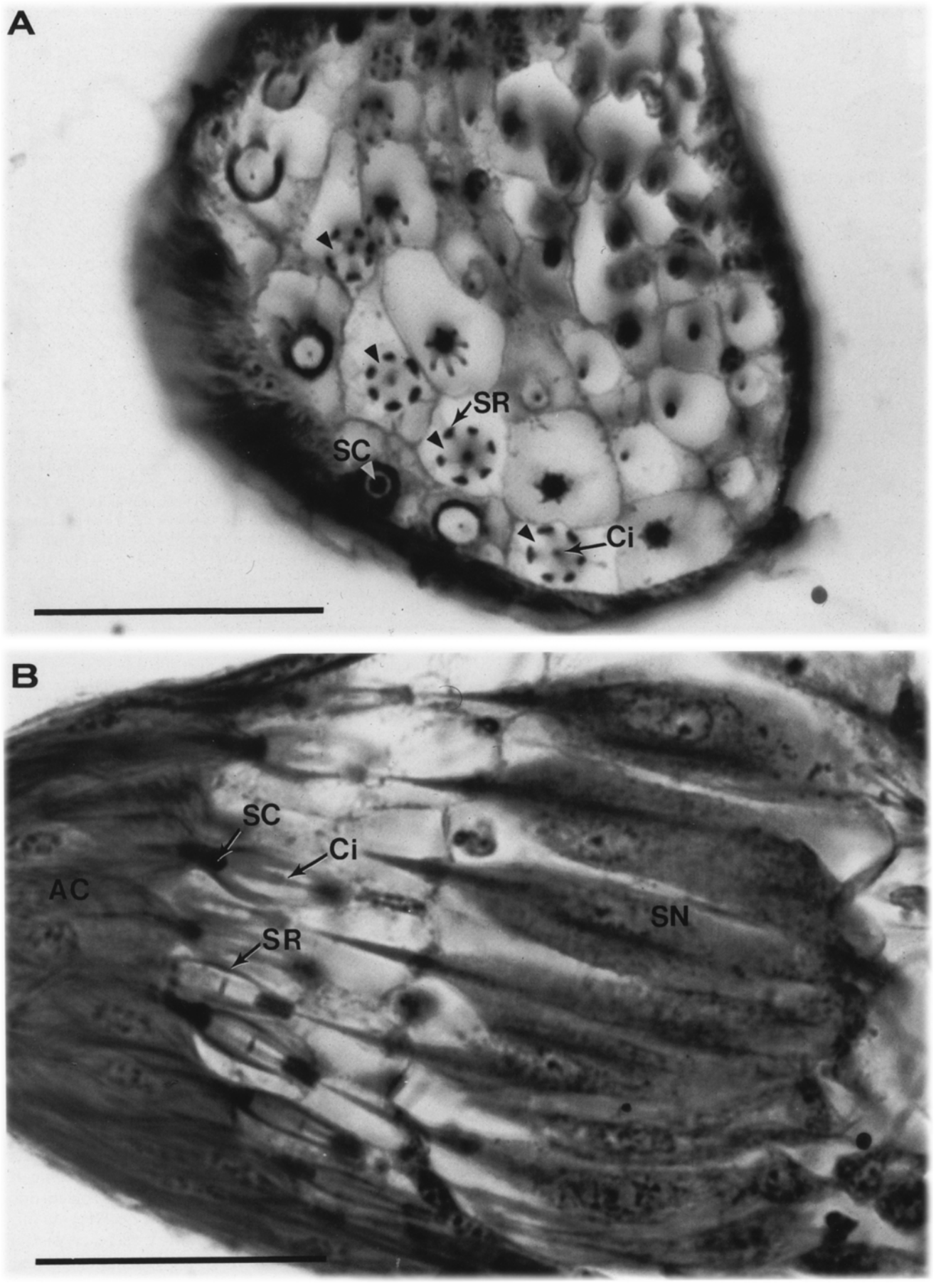
Fig. 6A,B. Horizontal longitudinal section of the thorax. The section shown in **A** is taken dorsally to the one schematically drawn in **B**. **A** Histological section showing the relative position of the prosternal chamber (*PC*) to the fused thoracic ganglia (*TG*) and the proximity of the bulba acustica (*BAc*). Note the thin (less than one micron) bilateral internal accessory membranes (*IAM*) connecting each *BAc* to the prosternal tympanal membranes (*PTM*). *Bar*: 100 μm. × 210. **B** Composite drawing showing that the bulba acustica is connected to the prosternal tympanal membrane (*PTM*) via the stiff auditory apodeme (*AA*) and the tympanal pit (*TP*). The auditory nerve (*AN*) innervating the bulba acustica is a branch of the prothoracic frontal nerve (*FN*). The auditory nerve runs along the suspensory ligament (*SL*) that links the *BAc* to the posterior boundary of the *PC*. The spiracular (*MSp*) and tracheal system (*T*) connect to the *PC*. *Ant* Anterior; *Lat* lateral. *Scale bar*: 100 μm

cells, the fibrous cell and the Schwann cell, which are closely associated with the sensory neuron (Fig. 8).

The photomicrographs in Fig. 9 are TEM transverse sections taken at the levels indicated by markers A to F in Fig. 8. In Fig. 9A the scolopale cap is surrounded by the ring formed by the distally fused scolopale rods. At the center of the cap is the cilium of the sensory cell (Fig. 9A, *arrow*). The sensory cilium presents the typical 9+0 microtubular organization of the axoneme (Fig. 9A, *inset*). Fig. 9B shows the ciliary dilation, with its somewhat different ciliary structure. The cilium is enlarged and the microtubules are closely apposed to the ciliary membrane. The center of the axoneme is filled in a laminated way with electron-dense material. In Fig. 9C, the scolopale rods are organized in an heptagonal arrangement surrounding the cilium, which, here also, contains nine peripheral pairs of microtubules, but no central pair (9+0). More proximally, the scolopale rods are closer and adjacent to the dendritic collar (Figs. 9D). The dendritic collar is an intracellular structure of the dendrite of the sensory neuron. Noteworthy are the laminated structure of the collar and the dendritic membranes extending between the scolopale rods (Fig. 9D). Slightly more proximally, in Fig. 9E, the dendritic collar and the scolopale rods, separated by the dendritic membrane, surround the ciliary root (Fig. 9E). The material composing the ciliary root does not show the typical axonemal microtubular structure. Fig. 9F depicts the dendrite of the sensory cell and the ciliary root surrounded by a Schwann cell. The region where the Schwann cell membrane apposes itself, the mesaxon, is clearly visible (Fig. 9F).

The innervation of the hearing organ

Each sensory organ is innervated by the frontal nerve, which extends from the fused thoracic ganglia anteriorly to the *BAc* (Fig. 10). Transverse sections stained with toluidine blue show that the frontal nerve enters the prothoracic ganglion slightly posteriorly to the prosternal nerve, a sensory nerve leading to the prosternal organ. The frontal nerve is both sensory and motor (Fig. 10A) and splits into two branches. One branch contains 8 large (3–5 μm) axons (Fig. 10B) and projects dorsally to innervate muscular tissue in the neck region (not shown). The other branch, the auditory nerve, consists of numerous small axons and enters the prosternal chamber to innervate the *BAc* (Fig. 10C). Fig. 10D shows the posi-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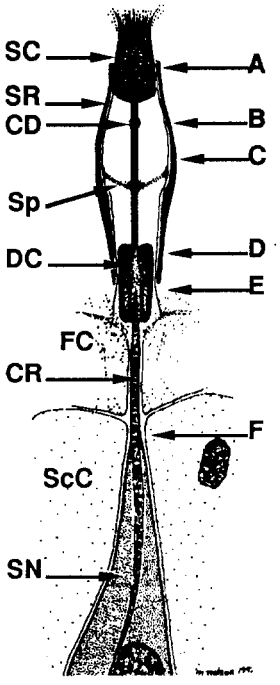

Fig. 8. Longitudinal reconstruction of a scolopidium based on light-microscopic longitudinal sections and transmission electron-microscopic (TEM) transverse sections. Letters on the right **A–F** refer to the corresponding transverse sections shown in Fig. 9. Scolopale cap (*SC*); scolopale rod (*SR*); ciliary dilation (*CD*); spoke (*Sp*); dendritic collar (*DC*); ciliary root (*CR*); fibrous cell (*FC*); sensory neuron (*SN*); Schwann cell (*ScC*)

tion of the cervical connectives, the prosternal nerve and the frontal nerve relative to the prosternal chamber.

The projection pattern of the primary auditory afferents was investigated by using a cobalt backfill technique. It appears that the pro-, meso-, and metathoracic neuropils, marked respectively T1, T2 and T3 in Fig. 10, receive the axonal projections from the sensory neurons from the *BAc*. The prothoracic neuropil displays the highest density of stained auditory terminals ($n=19$ preparations).

Discussion

The ormiine ear as a dipteran evolutionary innovation

In comparison with the hearing organs of other known acoustically active Diptera, the ormiine hearing organ is an evolutionary novelty. Acoustic signals have been widely reported in the mating behavior of mosquitoes (Mayer 1874; Roth 1948), a variety of drosophilid fruit flies (Bennet-Clark and Ewing 1967,1968; Hoikkala et al. 1989), and tephritid flies (Sivinsky and Webb 1986; Webb et al. 1983). In fruit flies, hearing mediates the reception of "love songs" that operate at short distances ranging from millimeters to a few centimeters, and have frequency spectral peaks of emission from 100 to 600 Hz, reflecting the wingbeat frequency of the calling male. In effect, this mating signal is transmitted and received in the acoustic near-field, in which air particles are set in motion by the beating wings. The hearing organ in these female flies is the Johnston's organ (Johnston 1855; Risler and Schmidt 1967; Boo and Richards 1975), a chordotonal organ located in the second segment of the antenna. The Johnston's organ detects movements of the antennal flagellum; the antenna may be covered with a dense "forest" of fibrillae (as in mosquitoes) or it may bear a feathery arista (as in drosophilids). In either case, the fibrillae or aristae are set into vibration by the signaler's wingbeat, in the acoustic near-field, which in turn excites the mechanoreceptor cells in the Johnston's organ. Interestingly, in ormiine flies, the arista is unbranched and naked, and this even constitutes a key taxonomic character of most tachinids (Wood 1987).

It is obvious that the acoustic behavior of ormiine flies falls outside the conventional dipteran pattern. In the first place, female ormiines are parasitoids that must locate their field cricket hosts at greater distances (meters to tens of meters). Thus, detection must occur in the acoustic far-field. Secondly, the calling song of the male cricket has a high frequency energy spectrum, from 3 to 8 kHz, depending on species (Walker 1986). These frequencies are one order of magnitude above the sensitivity of the Johnston's organ. Thus, to accommodate themselves to parasitizing crickets, female ormiine flies have to solve the same problem as female crickets: locate a male by hearing his calling song. By evolutionary convergence, ormiine flies evolved a tympanal organ that is sensitive to high frequency sounds at long distances from the sound source. As a consequence, the design features of the ormiine ear have more in common with a cricket's ear than a typical fly's ear.

The ormiine ear is a tympanal hearing organ

The ormiine tympanal organ possesses the three key characteristics of a tympanal organ: (1) it features a specialized thin cuticle, the tympanum; (2) this tympanum is contiguously associated with an air-filled cavity that is a specialized part of the tracheal system, and (3) the sensory, or chordotonal organ, is attached either to the tympanum or to an adjacent trachea. In the auditory insects described thus far, tympanal ears are notable for the diversity in their placement on the body. They are found on: the prothoracic tibiae of crickets and katydids (Schwabe 1906); the metathoracic segments of moths (Eggers 1919) and mantids (Yager and Hoy 1986); the wings of lacewings (Miller 1970); and the first or second abdominal segments of locusts (Gray 1960) and moths (Haskell and Belton 1957). In this paper, we report a novel location for a tympanal ear: the sternal complex of the prothorax.

Acoustic function of the prosternal membranes

Anatomical and physiological evidence shows that these tympanal membranes are involved in the reception of

Fig. 7A,B. Light-microscopy photomicrographs of the bulba acustica. Scolopale rods (*SR*); scolopale cap (*SC*); sensory cilium (*Ci*); sensory neuron (*SN*); attachment cells (*AC*). *Scale bars*: 10 µm, × 5400. **A** Transverse section through the region of the scolopidia. The *black arrowheads* indicate scolopidia sectioned at the same level. **B** Longitudinal, parasagittal section

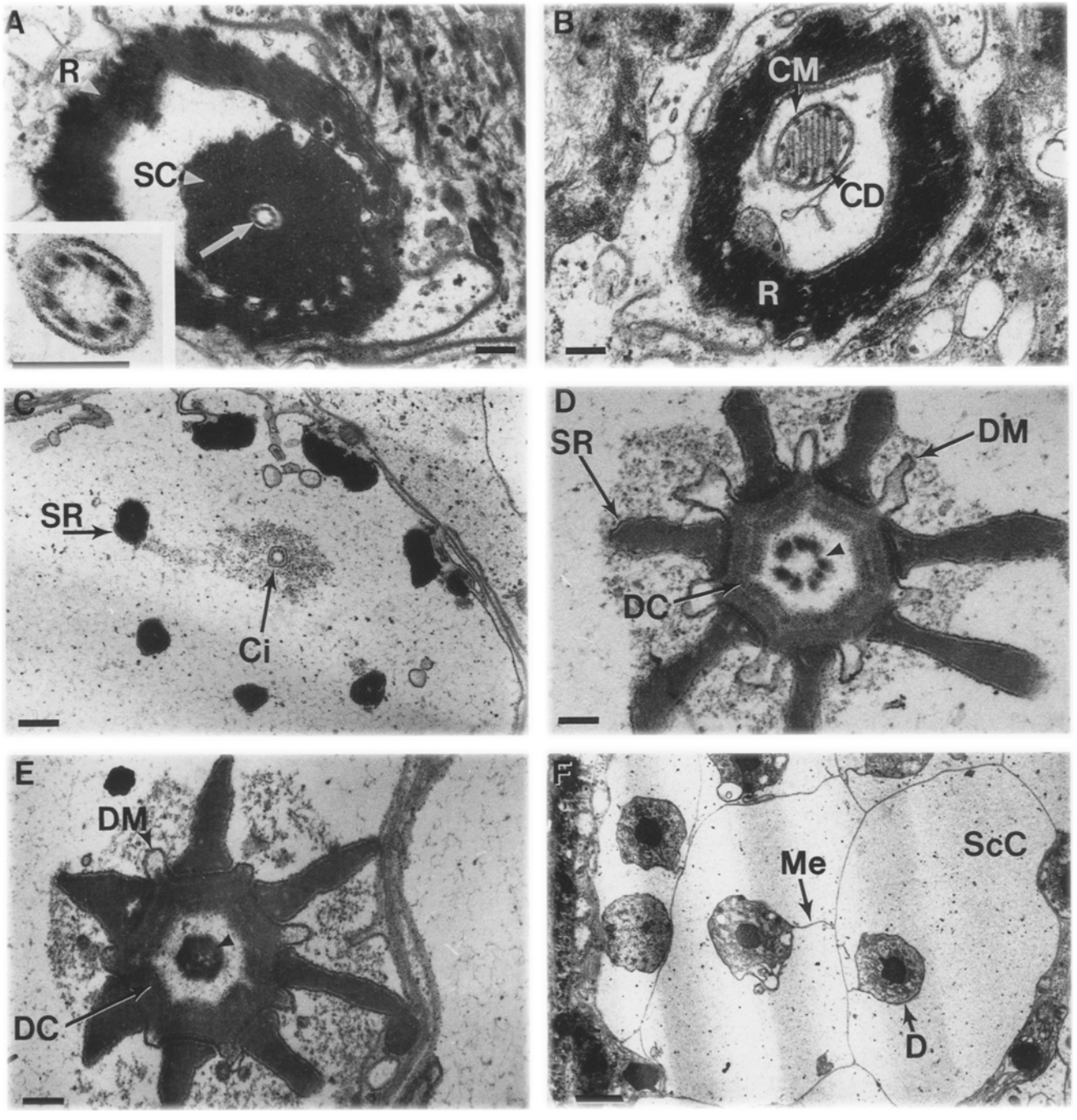

Fig. 9A–F. TEM photomicrographs of scolopidia in transverse section. Note: magnification for **C** and **F** is lower than for **A**, **B**, **D** and **E**. **A** At the level of the base of the scolopale cap. Ring of the fused scolopale rods (*R*). Scolopale cap (*SC*). The cilium of the sensory neuron (*arrow*) passes through the scolopale cap. *Scale bar*: 0.1 µm, × 66600. The *inset* shows the 9+0 axoneme of the sensory cilium. *Inset bar*: 0.1 µm, × 195700. **B** The scolopale rods are fused and form a ring (*R*) around the ciliary dilation (*CD*). The ciliary membrane (*CM*) surrounds the nine microtubules and the laminated material filling the center of the axoneme. *Scale bar*: 0.1 µm, × 66600. **C** Scolopale rods (*SR*) surrounding the sensory cilium (*Ci*) and the 9+0-type axoneme. *Scale bar*: 0.2 µm, × 33300. **D** Scolopale rods (*SR*) around the laminated dendritic collar (*DC*) and the central sensory cilium (*arrowhead*). The membrane of the dendrite (*DM*) protrudes between the scolopale rods. *Scale bar*: 0.1 µm, × 66600. **E** Transverse section through the middle of the dendritic collar (*DC*). Dendritic membrane (*DM*). The central structure, filled with dense amorphous material is the apex of the ciliary root (*arrowhead*), which lacks the 9+0 microtubular organization. *Scale bar*: 0.1 µm, × 66600. **F** Schwann cells (*ScC*) surrounding the dendrite (*D*) of the sensory neuron. (*Me*) mesaxon of the Schwann cell. The central structure of the dendrite is the ciliary root. *Scale bar*: 0.5 µm, × 16110

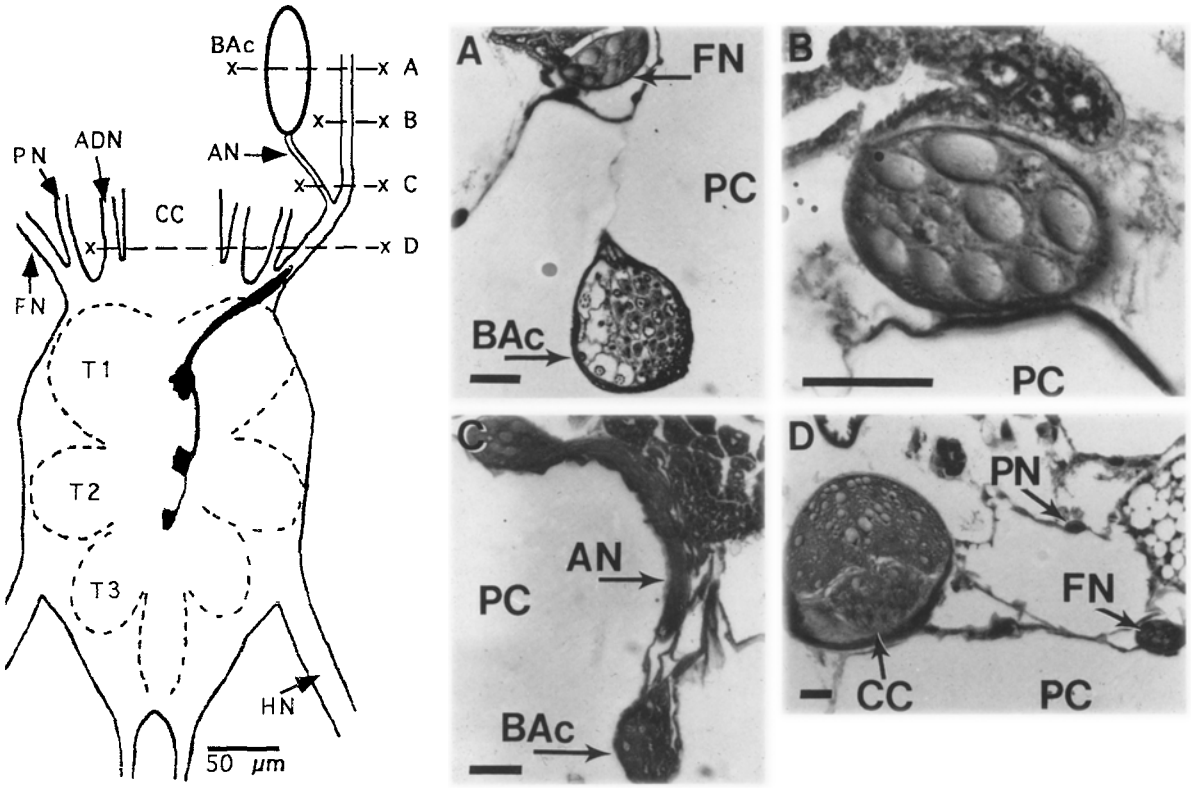

Fig. 10. Schematic drawing of the thoracic ganglion, the major anterior nerve roots, and the projections of the auditory afferents. Bulba acustica (*BAc*), auditory nerve (*AN*), frontal nerve (*FN*), prosternal nerve (*PN*), anterior dorsal nerve (*ADN*), cervical connectives (*CC*), haltere nerve (*HN*). The pro-, meso- and metathoracic neuropils, respectively (*T1*), (*T2*) and (*T3*), are indicated by the *dotted lines*. The projection of the auditory afferents is drawn from a camera lucida of a cobalt backfill. Letters on the right of the drawing (*A–D*) indicate the level of section corresponding to **A–D**. **A** Transverse section through the frontal nerve (*FN*) and the bulba acustica (*BAc*). Note the motor units of the frontal nerve and the scolopidia in the *BAc*. **B** Transverse section of the frontal nerve depicting the eight motor units that lead to thoracic neck muscles. **C** Transverse section of the frontal nerve as it splits into the motor tract and the auditory nerve (*AN*, cut obliquely). The *AN* is shown here about to connect to the *BAc*. The *AN* contains ~70 primary afferent fibers. **D** Transverse section showing relative positions of the cervical connective (*CC*), the prosternal nerve (*PN*) and the frontal nerve (*FN*). *PC* indicates the position of the prosternal chamber. *Scale bars*: 10 μm

air-borne sound waves (Lakes-Harlan and Heller 1992; Robert et al. 1992). Also, it has been shown that flies with their prosternal tympanal membranes (*PTMs*) punctured (but not impaired in their flight behavior) do not perform positive phonotaxis (Robert et al. 1992). Furthermore, electrophysiological recordings made from the neck connectives show that intact PTMs are necessary for acoustically evoked neural activity to occur. Hence, there is little doubt that the PTMs act as eardrums, collecting the sound energy and transducing it to mechanical energy to activate the scolopophorous sensory organs.

Tympanal hearing organs: the ormiine flies compared to other insects

As a tympanal organ, the ormiine ear bears some morphological similarity to the already diverse hearing organs found in Orthoptera, Homoptera, Lepidoptera, and Dictyoptera (Michelsen and Larsen 1985; Yager and Hoy 1987). However, the details of the morphology of the ormiine ear most closely approximate the ear of noctuid moths (Ghiradella 1971; Roeder 1967). With respect to their internal anatomy, the tympanal organs of both ormiines and noctuids are similar because: (1) the chordotonal sensory organ is directly attached distally to the tympanal membrane, (2) the chordotonal organ is attached proximally to the acoustic chamber by a "ligament" that exerts little or no tension on the chordotonal organ, and (3) the chordotonal organ is contained completely within the acoustic chamber. However, the ormiine tympanal organ uniquely differs from that of all other auditory insects in that both its left and right chordotonal organs are contained within the same air-filled acoustic chamber, rather than in the two separate, bilaterally symmetrical chambers. In other auditory insects, these chambers may be anatomically and acoustically isolated from each other, as in noctuid moths (Ghiradella 1971), or anatomically and acoustically coupled to each other, as in crickets, locusts, and cicadas (Michel 1974; Miller 1977; Young and Hill 1977). The arrangement in which both chordotonal organs are contained within a single chamber could severely constrain the reception of directional sound waves and, therefore, directional hearing.

Origin of the ormiine tympanal organ

The ormiine auditory apparatus appears to be derived from the prosternal sclerite and its associated membranes. The homology of the PTMs with the propleural air sacs has been recently proposed by Lakes-Harlan and Heller (1992) in another ormiine, *Therobia leonidei*. This hypothesis is in agreement with our observation of the prosternal complex in a related species of nonacoustic tachinid flies (*Myiopharus doryphorae*). These flies possess a flat and reduced prosternal triangular sclerite, which is associated with the prothoracic coxal membranes and covers the propleural air sacs.

In crickets, the subgenual organ of the prothoracic leg has been proposed to be the precursor of the mechanoreceptive structures of the tympanal ear (Meier and Reichert 1990; Michel 1974). In the ormiine fly, such a precursor could be a small chordotonal organ, made of a few scolopidia inserting in the region of the neck. This hypothesis is supported by the fact that a similar chordotonal organ in drosophilid and muscoid flies is innervated by the frontal nerve (Hertweck 1931; Hengstenberg 1991). In *O. ochracea* the frontal nerve innervates the auditory organ. It is, therefore, possible that the ormiine ear has derived its mechanoreceptive structures from a pre-existing neck proprioceptive chordotonal organ. Interestingly, in *Ormia*, as in *Calliphora* (Preuss and Hengstenberg 1992), the thoracic neck joint bears another proprioceptive mechanoreceptive organ, the prosternal organ. The mechanoreceptive units of the prosternal organ consist of campaniform sensilla.

The convergence in design

The general layout of the ormiine ear, including, respectively, the auditory apodeme and the tracheal openings is reminiscent of both the moth and cricket auditory apparatus. However, the possible involvement in auditory function of the large tracheal openings in *O. ochracea* has yet to be demonstrated.

A further convergent aspect of the hearing organ can be recognized at the level of the cellular organization. In all auditory insects, scolopidial cells have been found to be part of the mechanoelectric transduction process involved in the reception of sound waves. The ear of *O. ochracea* follows the same design principle by deploying approximately 65 to 75 scolopidia in the sensory organ, the bulba acustica (*BAc*). Examination of serial longitudinal sections of the whole *BAc* and transmission electron micrographs of scolopidia indicates that each scolopale cell is associated with only one bipolar neuron. Therefore, it seems that the number, for each BAc, of receptors dedicated to the function of hearing in *O. ochracea* amounts to 65 to 75 neurons. In comparison, in the mosquito *Culex pipiens*, the Johnston's organ has been reported to contain on the order of 20 000 scolopidia and associated bipolar neurons (Boo and Richards 1975).

It is also interesting that the fine structural features of the scolopidia show similarities with those previously described for other tympanate insects (Ghiradella 1971; Michel 1974; Yack and Fullard 1990; Moulins 1976 for review). In turn, mononematic and monodynal scolopidia seem to be ubiquitous features in the insect hearing organ (Yack and Roots 1992).

Sexual dimorphism, in structure and function

Sexual dimorphism of hearing organs is rare and has been reported in only a few vertebrate and invertebrate species (frogs: Narins and Capranica 1976; insects: Cardone and Fullard 1988; Yager 1990; Bailey and Römer 1991).

In *O. ochracea*, female and male hearing organs differ conspicuously in several respects. First, the female *PTM*s present a larger surface area than those of the male by a factor of 4.4 (see Table 2). Second, the surface area of the mesothoracic spiracular (*MSp*) openings in the female is half that in the male (Table 2). For females, the *PTM*: *MSp* ratio in surface area is of 4.8. In turn, the male hearing organ offers proportionately less tympanal than spiracular surface area (*PTM*: *MSp* ratio = 0.5).

Sexual dimorphism may reflect functional differences in hearing. Only females acoustically locate the singing cricket host to complete their reproduction. Indeed, not a single male has ever been attracted to (or even seen near) sound traps in the field (D. Robert, personal observation, T.J. Walker, personal communication). The anatomical dimorphism of the hearing organ has its physiological correlate in the frequency tuning of the ear. The best sensitivity of the females is very close to the carrier frequency of the host's song (Robert et al. 1992), whereas, for those frequencies, the male's sensitivity is reduced by some 40–50 dB. The male's best frequency is at 10 kHz, extending into the ultrasonic range where his sensitivity is similar to the female's. The function of hearing for males, as surmised by Robert et al. (1992), requires consideration of predation by aerial acoustic predators as well as possible courtship behavior involving acoustic signaling (Roeder 1967; Hoy 1989).

Convergent evolution: the case of hearing in insects

The hearing organ of *Ormia ochracea* is a tympanal organ. It exhibits the anatomical design features found in other tympanate insects, presumably reflecting the anatomical constraints imposed by the biophysics of high frequency hearing. However, within the pattern of generalized design features that characterize all insect tympanal ears, the ormiine ear displays several unique features that are described above. The ormiine ear presents an attractive opportunity for the study of the evolution of hearing organs in insects. As the first of its kind to be reported in the Diptera (Lakes-Harlan 1992; Robert et al. 1992), the ormiine ear permits a detailed analysis of the convergent evolution of tympanal organs in the different orders of insects; we have done this in the present report. In addition, an opportunity is presented to investigate convergent evolution *within* the Diptera since acoustic parasitism has also been reported within the Sarcophagi-

dae (Soper et al. 1976). While both ormiine tachinids and sarcophagids are calypterate flies (belonging, however, to different families and genera), both hear high frequency calling songs over long distances. Sarcophagids seek homopteran hosts (Soper et al. 1976) and possess modified prosternal structures (D. Robert, unpublished observations). Ongoing and future investigations promise to reveal information about the convergent evolution of tympanal ears within the order Diptera.

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