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ORIGINAL ARTICLE

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Evolution of the metathoracic tympanal ear and its mesothoracic homologue in the Macrolepidoptera (Insecta)

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Abstract Two independent methods of comparison, serial homology and phylogenetic character mapping, are employed to investigate the evolutionary origin of the noctuoid moth (Noctuoidea) ear sensory organ. First, neurobiotin and Janus green B staining techniques are used to describe a novel mesothoracic chordotonal organ in the hawkmoth, *Manduca sexta*, which is shown to be serially homologous to the noctuoid metathoracic tympanal organ. This chordotonal organ comprises a proximal scolopidial region with three bipolar sensory cells, and a long flexible strand (composed of attachment cells) that connects peripherally to an unspecialized membrane ventral to the axillary cord of the fore-wing. Homology to the tympanal chordotonal organ in the Noctuoidea is proposed from anatomical comparisons of the meso- and metathoracic nerve branches and their corresponding peripheral attachment sites. Second, the general structure (noting sensory cell numbers, gross anatomy, and location of peripheral attachment sites) of both meso- and metathoracic organs is surveyed in 23 species representing seven superfamilies of the Lepidoptera. The structure of the wing-hinge chordotonal organ in both thoracic segments was found to be remarkably conserved in all superfamilies of the Macrolepidoptera examined except the Noctuoidea, where fewer than three cells occur in the metathoracic ear (one cell in representatives of the Notodontidae and two cells in those of other families examined), and at the mesothoracic wing-hinge (two cells) in the Notodontidae only. By mapping cell numbers onto current phylogenies of the Macrolepidoptera, we demonstrate that the three-celled wing-hinge chordotonal organ, believed to be a wing proprioceptor, represents the plesiomorphic state from which the tympanal organ in the Noctuoidea evolved. This ‘trend toward simplicity’ in the noctuoid ear contrasts an apparent ‘trend toward complexity’ in several other insect hearing organs where atympanate homologues have been studied. The advantages to having fewer rather than more cells in the moth ear, which functions primarily to detect the echolocation calls of bats, is discussed.

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A. Introduction

With the exception of certain degenerate forms (Fullard et al. 1997), most members of the Noctuoidea possess physiologically sensitive tympanal ears on each side of the posterior metathorax (Fig. 1). The tympanal membrane is a thinned region of cuticle that forms part of the thoracic skeletal framework (Fig. 4b). A chordotonal organ, hereafter referred to as the tympanal organ, attaches to the inner surface of the membrane and contains either one (Notodontidae) or two (all other Noctuoidea) bipolar sensory cells (Eggers 1919). These cells respond best to high-frequency sounds (20–50 kHz) and are used to detect the echolocation calls of insectivorous bats and, in some cases, conspecific sounds (see, for example, Spangler 1988; Surlykke and Fullard 1989; Sanderford et al. 1998). When a moth hears a bat, a series of evasive flight maneuvers is initiated, which can allow it to escape predation (for reviews of hearing in the Noctuoidea, see Roeder 1974; Miller 1983; Spangler 1988; Fullard 1998).

All other Lepidoptera lack the external skeletal modifications associated with metathoracic ears (Forbes 1916; Richards 1932). In earlier studies, we described the structure and function of a chordotonal organ located at the base of the metathoracic wing in two atympanate species, *Manduca sexta* (Bombycoidea, Sphingidae) and *Actias luna* (Bombycoidea, Saturniidae) (see Yack and

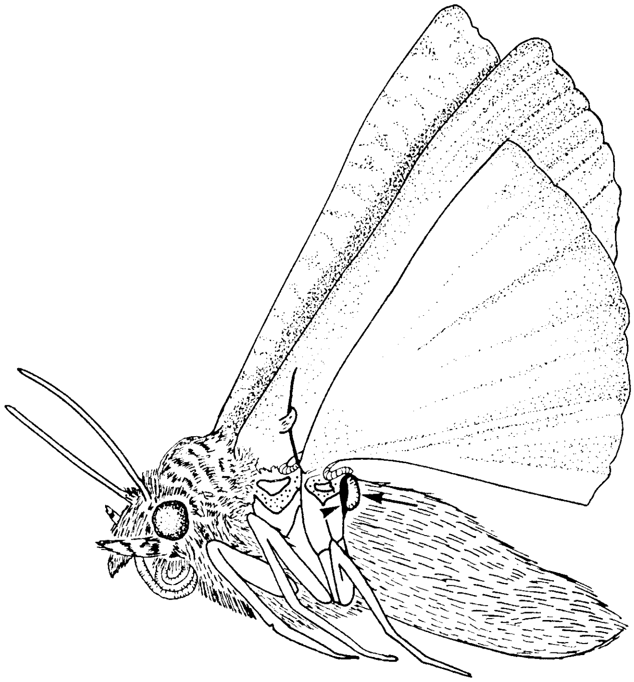

Fig. 1 Lateral view of a noctuid moth, indicating the location of the tympanic cavity (*arrowhead*) and the abdominal hood (*arrow*) covering the cavity. The tympanic membrane is situated within the cavity, set within the skeletal framework of the posterior metathorax

Fullard 1990; Yack 1992; Yack and Roots 1992). In both cases, the atympanate chordotonal organ has three bipolar sensory neurons, attaches to a non-tympanal membrane or cuticle ventral to the axillary cord of the metathoracic wing, and appears to function as a proprioceptor detecting vibrations and movements of the hind-wing. It was suggested that the three-celled wing-hinge chordotonal organ represents the plesiomorphic, preauditory state and that, through evolutionary modifications of peripheral structures, the chordotonal organ changed functionally from a proprioceptor to a hearing organ. The hypothesis of the proprioceptive origin of lepidopteran tympanal sensilla (von Kennel and Eggers 1933), however, has never been tested systematically.

Recent studies have demonstrated the value of studying the evolution of behavioral and morphological traits by mapping these traits onto existing phylogenies of large taxa (see, for example, Brooks and McLennan 1991; Gwynne 1995), but few have employed phylogenetic analyses to learn about the evolution of insect nervous systems (see, for example, Shaw and Meinertzhagen 1986; Edgecomb et al. 1995; Strausfeld et al. 1998). The evolutionary origin of moth hearing organs can be conveniently studied using this method, since the ears (and their proposed atympanate homologues) consist of very few cells, whose presence or absence can be easily quantified.

In the present study, we use two comparative methods to examine the evolution of the metathoracic ear in the Macrolepidoptera, serial homology and phylogeny. We examine a previously undescribed chordotonal organ, and proposed serial homologue of the tympanal organ, in the atympanate mesothorax. We then examine tympanal organs and/or their thoracic homologues in 23 species representing seven ditrysian superfamilies [six from the present study and one from a previous study (Hasenfuss 1997)], and map the evolution of chordotonal organ cell numbers using a recent phylogeny of the Macrolepidoptera (Kristensen and Skalski 1998).

B. Materials and methods

I. Taxa examined

Table 1 lists the species of Lepidoptera examined in this study. Individuals were obtained from four different sources:

1. *Actias luna* (Linné, 1758), *Antheraea polyphemus* (Cramer, 1776), *Samia cynthia* (Drury, 1773), *Hyalophora cecropia* (Linnaeus, 1758), *Manduca sexta* (Linné, 1763), and *Vanessa cardui* (Linné, 1758) pupae were purchased from a commercial supplier (North Carolina Biological, Burlington, N.C., USA).
2. *Lirimiris meridionalis* Schaus, 1904, one Geometroidea[1], and *Oxytenis* spp. were collected in the field in Panamà.
3. *Prionoxystus robiniae* (Peck, 1818), *Pantographa limata* (Grote and Robinson, 1867), *Callosamia promethea* (Drury, 1773), *Tolype laricis* (Fitch, 1856), *T. velleda* (Stoll, 1791), *Smerinthis jamaicensis* (Drury, 1773), *Biston betularia* (Linné, 1758), *Catocola coccinata* Grote, 1872, *Pyrrharctia isabella* (J.E. Smith, 1797), *Lymantria dispar* (Linné, 1758), *Heterocampa obliqua* Packard 1864, *Nadata gibbosa* (J.E. Smith, 1797), and *Peridea angulosa* (J.E. Smith, 1797) were collected from black-lights at the Queen's University Biology Station (Leeds County, Ontario, Canada).
4. *Trichoplusia ni* (Hübner [1803]) were provided by Dr. J. Myers from the Department of Zoology, University of British Columbia.

II. Anatomical nomenclature

Anatomical nomenclature used for muscles and nerves follows that of Nüesch (1953, 1957) and Yack and Fullard (1990). Nomenclature relating to tympanal structures was taken from Eggers (1919) and Treat (1959), and terms describing the atympanate meso- and metathoracic skeletal structures, from Eaton (1988) and Nüesch (1953).

III. Dissection and anatomical procedures

A sagittal dissection approach was used to describe the peripheral projections of the mesothoracic IIN1 nerve in *M. sexta*. Before each dissection, the moth was cooled for 30 min. Once cooled, both the head and abdomen were removed, and the descaled thorax was cut in half in a sagittal plane and pinned to Sylgard (Dow Corning, Midland, Mich., USA) in a petri dish. Drawings of the mesothoracic nerve branch IIN1 were carried out by staining and following the whole branch from the ganglion to the periphery with a 0.05% solution of Janus green B in saline (Paul 1974). The branches of IIN1b were traced as closely as possible to their target muscles or tissues and drawn with the aid of a Wild (M3) dissection microscope and camera lucida attachment, or photographed.

Since Janus green B stains only the whole nerve branch leading to the chordotonal organ (Yack 1993), it was necessary to employ a second staining method to determine the number of sensory

[1] This individual was identified as a Geometroidea based upon the external skeletal characteristics of the abdominal ear

Table 1 Survey of the number of scolopidia (based on number of scolopale caps) in Noctuoidea tympanal chordotonal organs and wing-hinge chordotonal organs of atympanate Lepidoptera. Numbers in parentheses indicate data taken from other studies. (–: Data not available)

		Species[a]	Metathorax, number of caps	Mesothorax, number of caps
Yponomeutoidea	Yponomeutidae	*Yponomeuta plumbellus*	(3)	(3)
Cossoidea	Cossidae	*Prionoxytus robiniae*	3	3
Pyraloidea	Pyralidae	*Pantographa limata*	3	3
Bombycoidea	Saturniidae	*Actias luna*	3	3
		Antheraea polyphemus	3	–
		Callosamia promethea	3	3
		Samia cynthia	3	–
		Hyalophora cecropia	3	3
	Oxytenidae.	*Oxytenis spp*	3	3
	Sphingidae	*Manduca sexta*	3	3
		Smerinthis jamaicensis	3	–
	Lasiocampidae	*Tolype laricis*	3	–
		Tolype velleda	3	–
Papilionoidea	Nymphalidae	*Vanessa cardui*	3	3
Geometroidea	Geometridae	*Biston betularia*	3	3
	?	Panamanian	3	–
Noctuoidea	Noctuidae	*Catocola coccinara*	(2)	3
	Arctiidae	*Pyrrharctia isabella*	(2)	3
	Lymantriidae	*Lymantria dispar*	(2)	3
	Notodontidae	*Lirimiris meridionalis*	(1)	2
		Heterocampa obliqua	(1)	2
		Nadata gibbosa	(1)	2
		Peridea angulosa	(1)	2

[a] A minimum of two individuals (one male and one female) were examined for each species (except for *P. robiniae*, since only males were available at the time of the study)

neurons in the chordotonal organ. This was done by filling the chordotonal nerve branch (IIN1) to the periphery with neurobiotin (Vector Laboratories, Burlingame, Calif., USA). After the thoracic nervous system had been exposed by a dorsal dissection approach (Yack and Fullard 1990), the IIN1b branch was severed at the point where it attaches to IIN1, and its cut end was placed in a 'boat' (a small disc of Parafilm surrounded by a rim of silicon grease) filled with distilled water. After approximately 30 s the distilled water was replaced with 10% neurobiotin dissolved in distilled water. The boat and any exposed tissues were covered with silicon grease in order to prevent dehydration. The preparation was then placed in a partially sealed container (lined with moistened paper towels in order to maintain a high humidity) and kept at 4°C for 4–7 h. The chordotonal organ and its attached nerve branch were then dissected out of the moth and pinned to a small petri dish lined with Sylgard. The chordotonal organ was fixed for 3 h in chilled 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Following fixation, the preparation was rinsed 3×10 min in buffer, incubated overnight in Vector kit ABC solution (9.9 ml 0.25% Triton X in PBS and 30 µl each of solutions A and B), and rinsed 2×15 min in buffer. The nerve cells were then visualized by reacting the chordotonal organ in a solution of diaminobenzidine (10.0 mg diaminobenzidine, 18.98 ml phosphate buffer, 0.56 ml 1% cobalt chloride; 0.40 ml 1% nickel ammonium sulfate, and 0.06 ml 0.1% distilled water). The reaction was monitored for about 10 min until a black precipitate was observed in the solution. The diaminobenzidine solution was then replaced with buffer, and the chordotonal organ rinsed 3×10 min in buffer. The chordotonal organ was then dehydrated in ethanol, cleared in methyl salicylate, mounted in Permount, and photographed.

In a taxonomic survey of atympanate chordotonal organs, the number of scolopidia in each organ was determined by counting the number of scolopale caps. Chordotonal organs of both the meso- and metathorax were located by following the IIN1b or IIIN1b nerve branches with Janus green B (described above). The chordotonal organ was lightly stained with Janus green B, carefully extracted from the animal, placed on a microscope slide in a drop of saline, cover-slipped, and viewed with a compound microscope. Scolopale caps were easily identified, counted, and photographed.

IV. Scanning electron microscopy

Atympanate wing-hinge regions and tympanal membranes were fixed with buffered fixative (0.25% glutaraldehyde and 4% formaldehyde) for 2–4 h at 5°C, and then treated with hexamethyldisilazane (Nation 1983). The tissue was then air-dried, sputter-coated with gold, and examined with a Hitachi S2500 scanning electron microscope.

V. Phylogeny

For our phylogenetic analysis we have used the Macrolepidoptera section of the Lepidoptera phylogeny presented in Kristensen and Skalski (1998) and the Noctuoidea phylogeny summarized in Weller et al. (1994). To these phylogenies we have applied the taxonomic affiliations of the species analyzed and the character states of the number of chordotonal sensory cells present in the meso- and metathoracic segments.

C. Results

I. General structure of the mesothoracic chordotonal organ in *M. sexta*

The mesothoracic IIN1 branch arises from the dorsal surface of the connective between the pro- and pterothoracic ganglion (Fig. 2b). The first branch (IIN1a) emerges from the base of IIN1, continuing anteriorly to innervate Iis, a short intersegmental muscle located between the prothorax

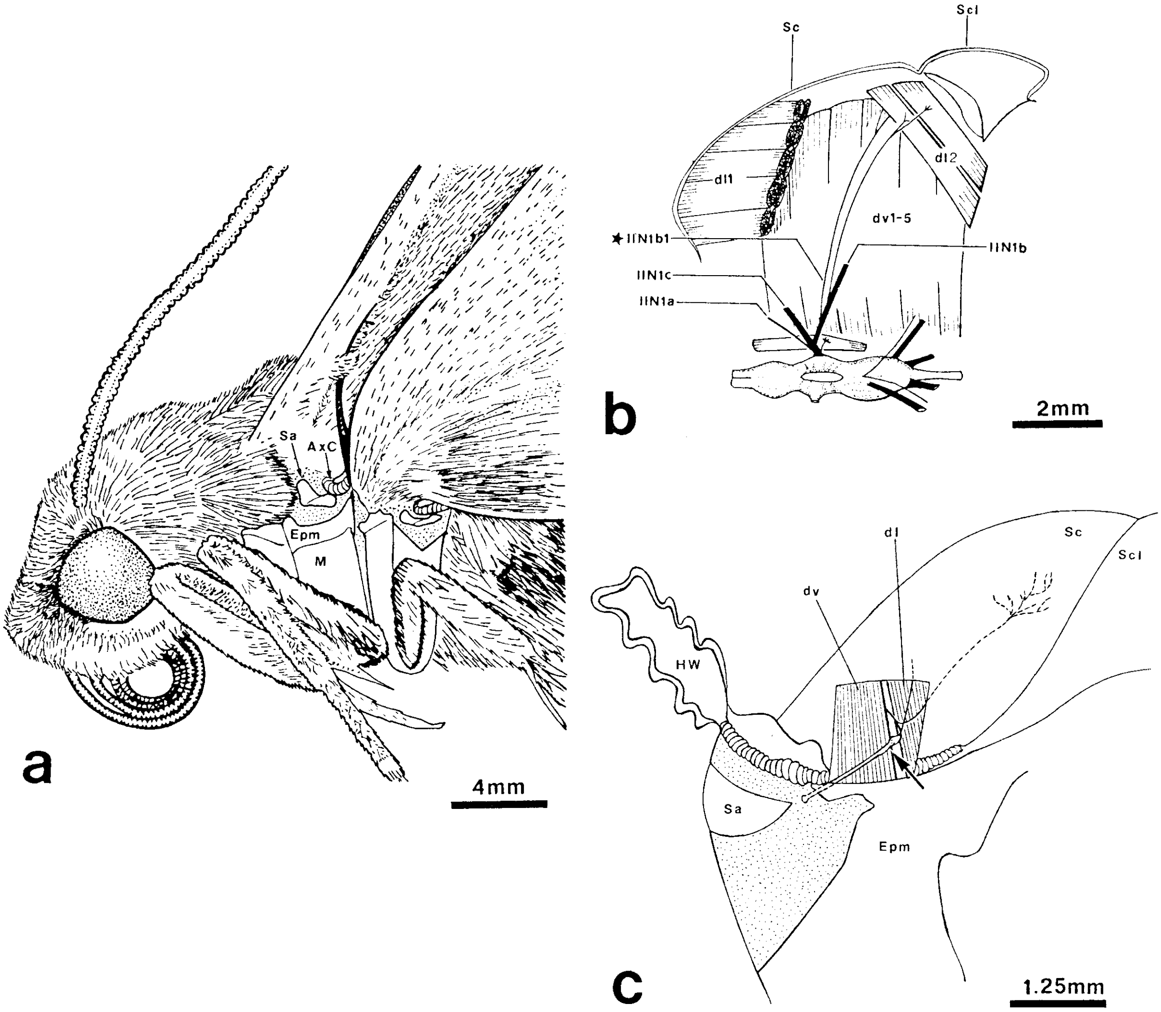

Fig. 2a–c Diagrams illustrating the peripheral projections of mesothoracic nerve branch IIN1b1 (tympanal nerve homologue) in *Manduca sexta*. **a** Lateral view of the descaled thorax. The subalar sclerite (*Sa*), axillary cord (*AxC*), epimeron (*Epm*), and meron (*M*) of the mesothorax are labeled. **b** Right half of the mesothorax viewed from the midline, showing the principal branches of nerve root IIN1 with surrounding nerves and musculature. The first branch of *IIN1b*, *IIN1b1* (*star*), takes a lateral course between the dorsoventral and dorsolongitudinal musculature toward the mesothoracic wing-hinge. **c** Posterior view of the left, descaled mesothorax with parts of the scutum and tracheal tissue removed to reveal the peripheral projections of the IIIN1b1 chordotonal organ. The strand of the chordotonal organ attaches to the membranous cuticle just medial to the subalar plate. A second branch continues dorsally and medially, branching beneath the dorsal rim of the mesothoracic scutum. *dl* Dorsolongitudinal musculature, *dv* dorsoventral musculature, *HW* hind wing, *Sc* scutum, *Scl* scutellum

and the mesothorax. The next branch that leaves the main nerve is IIN1b. It is directed dorsoposteriorly and innervates the large dorsolongitudinal flight muscles through its second and main branches. The first branch that leaves IIN1b, named IIN1b1, climbs dorsally and posteriorly between the dorsolongitudinal and dorsoventral musculature toward the mesothoracic wing-hinge. IIN1b1 divides into two branches: one climbs dorsally, where it branches beneath the mesothoracic scutum and the other (named the penultimate branch; see Yack and Fullard 1990) follows a ventral and posterior course through a non-expanded region of tracheal tissue, terminating in a chordotonal organ (Fig. 2c, 3a). The organ is suspended at its proximal end to the mesothoracic scutum by a ligament and at its distal end it attaches to an undifferentiated region of membranous cuticle ventral to the axillary cord extending medially from the subalar plate to the sclerotized region of the epimeron (Fig. 2a,c, 4c). A putative second branch of the penultimate was occasionally observed in Janus green B preparations, but its attachment site was not determined.

The mesothoracic chordotonal organ comprises a proximal scolopidial region (approximately 65 µm in diameter and 160 µm in length) and a distal attachment strand (approximately 1500 µm in length). Neurobiotin staining reveals three bipolar sensory neurons in the scolopidial region (Fig. 3b). The orientation and appearance of the neurons are similar to the metathoracic wing-hinge chordotonal organs previously described in *A. luna* and *M. sexta* (see Yack and Fullard 1990; Yack 1992; Yack and Roots 1992) in that each cell's dendrite possesses a distal dilation and terminates as a dendritic cilium at the distal end of the scolopidial region (Fig. 3b). The termi-

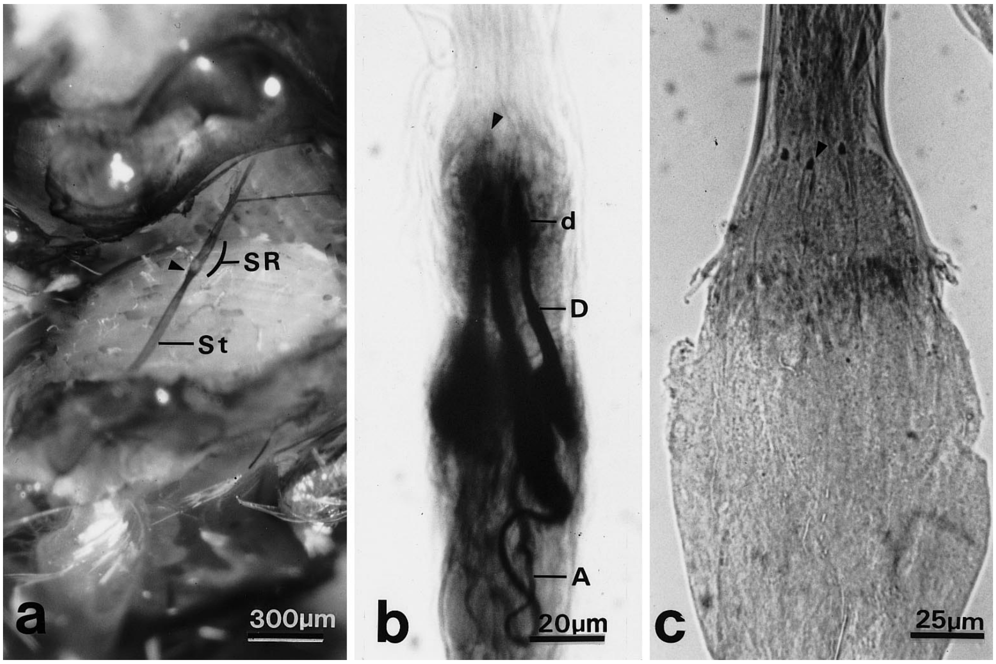

Fig. 3a–c Light micrographs of the mesothoracic wing-hinge chordotonal organ in *M. sexta*. **a** Dissection of the posterior mesothoracic wing-hinge, exposing the Janus green B-stained chordotonal organ. An *arrowhead* points to the distal end of the scolopidial region (*SR*), where the scolopale caps are located. *St* Chordotonal organ strand. **b** Whole mount of the three bipolar sensory neurons stained with neurobiotin. An *arrowhead* points to the position of one scolopale cap located at the distal end of a dendritic cilium. *A* Axon, *d* dilation of dendrite, *D* dendrite. **c** Whole mount of the scolopidial region stained with Janus green B. An *arrowhead* points to one of the three scolopale caps

nal tip of the dendritic cilium is associated with a single scolopale cell and cap (Fig. 3c).

II. Comparisons of thoracic chordotonal organs in representatives of seven superfamilies

Table 1 describes the results of a survey of thoracic chordotonal organ cell numbers. A mesothoracic chordotonal organ was located in all seven superfamilies. In each case, the chordotonal organ is innervated by the same nerve branch (the penultimate branch of IIN1b1) as that described in *M. sexta*. In all groups except for the Notodontidae, the mesothoracic chordotonal organs are structurally similar to one another. Each has three sensory cells and a long distal attachment strand that attaches to the posterior metathoracic wing-hinge region. The exact site of attachment varies only slightly. In *V. cardui* the strand attaches to the lateral edge of the sclerotized cuticle of the epimeron, while in all other cases, it attaches to the soft membrane slightly lateral to the epimeron. The notodontids were the only group that showed variation from the three-celled condition. Only two sensory cells were observed and the attachment strand was consistently short (bringing the scolopidial region very close to the membranous attachment site) compared to all other chordotonal organs examined.

The metathoracic chordotonal organs of all atympanate groups were similar in their general structure and appearance, each having three sensory cells and a distal attachment strand resembling that described above in the mesothorax of *M. sexta*. In the Sphingidae and Papilionidae, there was slight variation in the chordotonal organ strand attachment site: in the Sphingidae, it attaches to the sclerotized cuticle of the epimeron and is manifested externally by a non-articulating, peg-like structure (Fig. 4d). Scanning electron microscope examinations of these structures revealed no apparent pores or sensory structures (other than the chordotonal organ) associated with this peg. When observed internally, this structure appeared as a slight indentation of the cuticle, and the chordotonal organ strand attached to the inner part of this indentation. In *V. cardui*, the chordotonal organ strand attaches to the sclerotized epimeron, as it does in the mesothorax of this species.

III. Phylogenetic analyses of thoracic chordotonal organ cell numbers

Figure 5 illustrates the combined phylogenies of Kristensen and Skalski (1998) and Weller et al. (1994) that we use to map our data of chordotonal organ cell number. Figure 6a describes the character evolution of the mesothoracic chordotonal organ cell number and indicates that three cells are the plesiomorphic condition for this organ. Figure 6b maps the evolution of the metathoracic chordotonal organ (with the presence of the metathoracic ear in the Noctuoidea) and indicates that, as with the mesothoracic chordotonal organ, three cells are the plesiomorphic condition. In both thoracic chordotonal organs it appears that there has been a reduction of cell numbers with the greatest reduction being present in the Notodontidae with only a single sensory cell present in the ears of this family. Considering the unresolved relationship of the Notodontidae to the rest of the Noctuoidea, however, we cannot determine if the one-celled tympanal organ evolved from the noctuoid two-celled tympanal organ or the atympanate three-celled chordotonal organ (see Discussion).

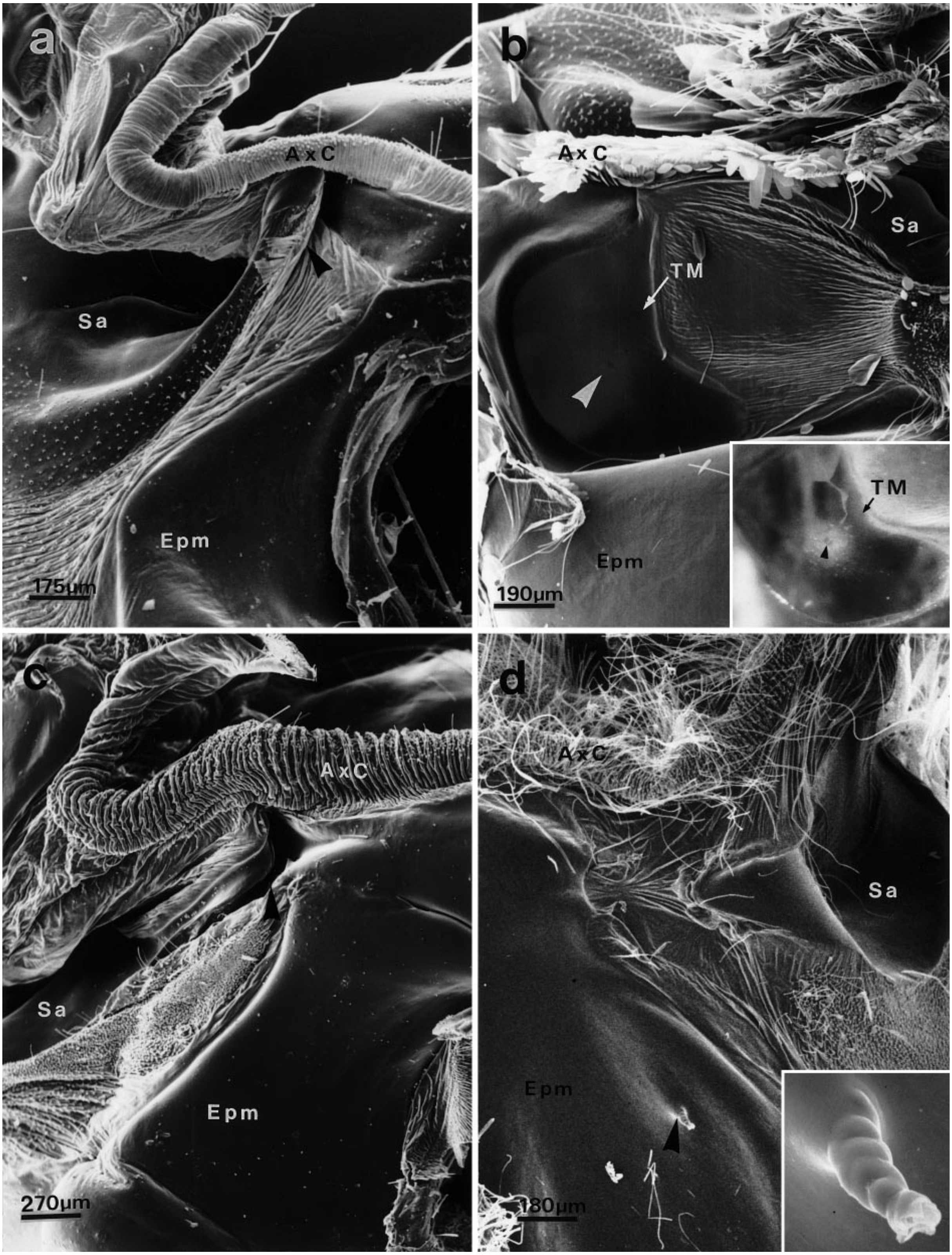

Fig. 4a–d Posterior views of the meso- and metathoracic wing-hinges of a tympanate and an atympanate moth. **a** Mesothorax of *Trichoplusia ni* (Noctuoidea). An *arrowhead* marks where the chordotonal organ strand attaches internally to the subalar membrane. *AxC* Axillary cord, *Epm* epimeron, *Sa* subalar sclerite. **b** Right metathorax of *T. ni* showing the tympanal membrane. An *arrowhead* marks where the tympanal organ strand attaches to the tympanal membrane. *TM* Tympanal membrane. The *inset* is a light micrograph of the tympanal membrane. The *arrowhead* shows where the tympanal organ attaches to the center of the tympanum. **c** Right mesothoracic wing-hinge of *M. sexta* (Bombycoidea). An *arrowhead* marks the attachment site of the chordotonal organ strand. **d** Right metathorax of the *M. sexta*, with the wing stretched in an upward position. An *arrowhead* marks the region where the chordotonal organ strand attaches internally to the sclerotized cuticle of the epimeron. A peg-like structure (enlarged in the *inset*) marks the attachment site

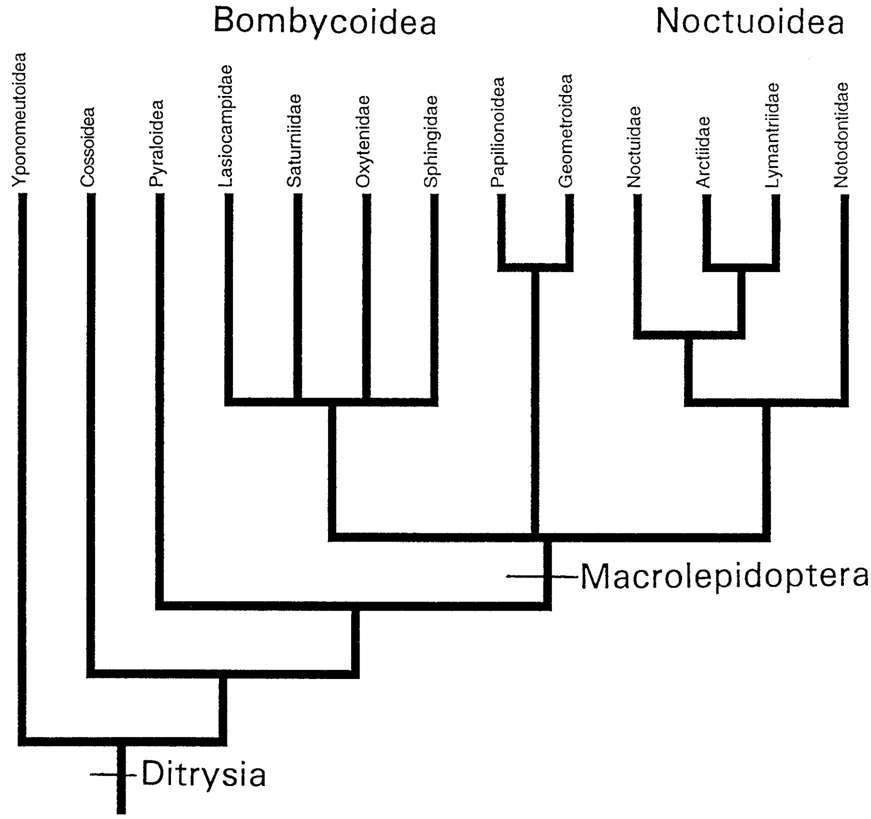

Fig. 5 A combination of the phylogenies for the Ditrysia (Kristensen and Skalski 1998) and the Noctuoidea (Weller et al. 1994) using the superfamilies surveyed in the present study. According to Lemaire and Minet (1998) the Oxytenis group is a subfamily (Oxyteninae) in the Saturniidae rather than a separate family, and the Lasiocampidae are the sister group of the Bombycoidea in the strict sense although this does not alter the interpretations in our discussion (N.P. Kristensen personal communication)

D. Discussion

We conclude that the metathoracic ear of the Macrolepidoptera evolved from a three-celled wing-hinge chordotonal organ, from two lines of evidence: (a) anatomical comparison of the tympanal organ with a proposed segmental homologue and (b) a phylogenetically based character analysis of tympanate and atympanate thoracic chordotonal organs.

I. Serial homology

In segmented animals, one way of exploring evolutionary changes to the nervous system has been to compare homologues (i.e., equivalent structures) in serial segments of the same animal (see, for example, Wilson and Hoyle 1978; Davis 1983; Rossler 1992; Boyan 1998). Although serial homologues are not homologues in the strict sense of the word (i.e., two structures that could, in principle, be traced back to a common ancestral precursor; see Campbell and Hodos 1970) they are presumably ontogenetically derived from similar precursors and, therefore, differences between segments may reflect evolutionary changes acting on the specialized member of the series (Dumont and Robertson 1986).

First, we provide evidence to suggest that the mesothoracic chordotonal organ is a segmental homologue of the noctuoid tympanal organ. One method researchers have used in establishing homology between neural structures has been to identify similarities in their morphology and in their relationships to homologous peripheral structures of the musculature or exoskeleton (see Campbell and Hodos 1970; King and Valentino 1983). Since the present study is the first to describe a mesothoracic wing-hinge chordotonal organ in the Lepidoptera, we will describe the similarities between the tympanal IIIN1 and the atympanate IIN1 nerves.

The general nerve pattern of IIN1 in *M. sexta* (present study) reflects that of the Noctuoidea nerve (IIIN1), which has been described in detail elsewhere (Roeder and Treat 1957; Treat 1959; Paul 1973; Yack and Fullard 1990). The most significant difference between the two nerves occurs in the penultimate branch of IIIN1b1 and IIN1b1. The noctuoid metathoracic penultimate branch passes through an expanded air-filled cavity (the tympanic air sac) which leads to the tympanic membrane. Before reaching the membrane, it runs along an apodemal infold [known as the “Bügel”, which comprises part of the specialized skeletal framework associated with the tympanum (Eggers 1919)]. At this attachment site a large multiterminal neuron, the B cell, occurs[2]. The nerve continues toward the tympanic membrane, where it terminates as a chordotonal organ [with two (or one in notodontids) bipolar sensory cells] and attaches distally to the tympanic membrane by a short, rigid attachment strand. In comparison, the mesothoracic penultimate

[2] In the Notodontidae the “Bügel” is absent and the B cell attaches to the wall of the tympanic cavity (Surlykke 1984)

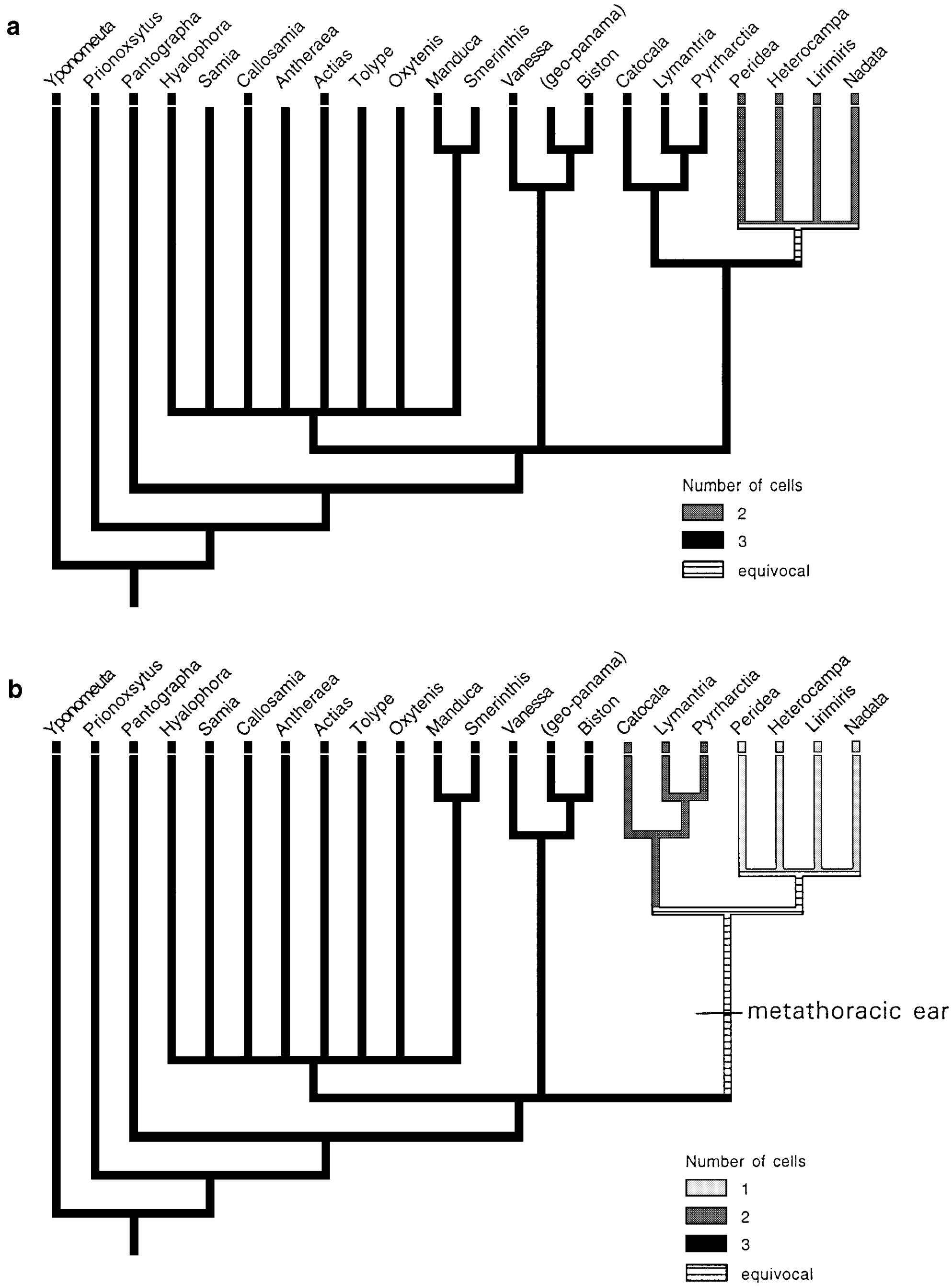

Fig. 6. a Proposed evolution of the number of sensilla in the mesothoracic chordotonal organ using the phylogeny of Fig. 5. **b** Proposed evolution of the number of sensilla in the metathoracic chordotonal organ including the evolution of the metathoracic ear in the Macrolepidoptera. For both maps, the outgroups are the Pyraloidea, Cossoidea, and Yponomeutoidea [data for the yponomeutoid from Hasenfuss (1997)]

branch in *M. sexta*, associated with no specialized tracheal enlargements or modified skeletal structures, simply ends in the long, three-celled chordotonal wing-hinge receptor. Although we did not observe a multipolar cell directly in the neurobiotin preparations (because we were dissecting out the chordotonal organ only), the putative second branch of the penultimate may prove to be a multipolar cell. Further investigation is necessary before we can conclude the presence or absence of a mesothoracic homologue of the B cell.

The general anatomy of the mesothoracic chordotonal organ and its peripheral attachment site reflects that of the atympanate metathoracic chordotonal organ previously described in *M. sexta* and *A. luna* (see Yack and Fullard 1990; Yack and Roots 1992; Yack 1993). Richards (1932) has drawn homologies between the skeletal structures of the external metathoracic winghinge in atympanate Lepidoptera and the Noctuoidea with specialized tympana. He suggests that the tympanal membrane itself resulted from either a reduction of the subalar membrane or the adjacent sclerotized cuticle of the epimeron, to the extreme thinness of a tympanic membrane. The present study of both the meso- and metathoracic chordotonal organs, together with previous studies of metathoracic chordotonal organs, show that the atympanate chordotonal organs attach to these exact regions that Richards proposes to be pretympanal sites.

Based on the anatomical similarities of both neural and skeletal structures associated with the chordotonal organs of the ear in Noctuoidea and the atympanate mesothoracic wing-hinge, we propose serial homology between these structures. We judge that the wing-hinge chordotonal organ, being associated with an unspecialized atympanate wing membrane, represents the pretympanal condition. The following discussion lends phylogenetic support to this observation.

II. Phylogeny of chordotonal organ cell numbers

The phylogenetic examination of cell numbers in the meso- and metathoracic segments indicates that the plesiomorphic state of the chordotonal organ in both of these segments is three-celled. The analysis further indicates that in the mesothorax this chordotonal organ has largely remained as a three-celled wing-hinge proprioceptor, but that in the metathorax it has diverged into auditory and non-auditory states. In atympanate forms, the metathoracic chordotonal organ has remained a three-celled proprioceptor, presumably providing information about wing position and/or vibration (Yack and Fullard 1990), but in the Noctuoidea it has become a two (or one)-celled auditory chordotonal organ. There is general agreement among most phylogenies and classifications of the Lepidoptera that the Noctuoidea are a lineage derived from atympanate forms (see Brock 1971; Robbins 1987; Kristensen and Skalski 1998) and that metathoracic tympana have never been reported in any non-noctuoid Lepidoptera. The external metathoracic structures appear unmodified and uniform throughout the atympanate forms (Forbes 1916; Eggers 1919; Richards 1932). In agreement with these observations, we find little or no variation in the general structure and location of the wing-hinge chordotonal organ (of both the meso- and metathorax) among atympanate Lepidoptera, including the “lower” Cossoidea and Yponomeutoidea.

Larval chordotonal organs, proposed to be developmental precursors of the tympanal organ in a noctuoid moth (*Lymantria dispar*; see Lewis and Fullard 1996) or the tympanal organ homologue in two earless moths [*Malacosoma disstria* Hübner, (1820), *Yponomeuta plumbellus* (Denis and Schiffermüller, 1775); see Lewis and Fullard 1996; Hasenfuss 1997], also possess three sense cells. In the atympanate condition this chordotonal organ is reported to remain relatively unchanged from the larval to the adult condition (Hasenfuss 1997). These studies provide further support to the argument that the plesiomorphic condition is three-celled.

III. An evolutionary trend toward neural simplicity?

Numerous anatomical and physiological studies have concluded that the Notodontidae possess one auditory nerve cell (anatomy: Eggers 1919; Surlykke 1984, physiology: Fullard 1984; Surlykke 1984; Fullard et al. 1997) and that all other Noctuoidea possess two cells (anatomy: Eggers 1919; Ghiradella 1971; Surlykke 1984; Coro and Perez 1987, physiology: see Roeder 1974). Assuming that the three-celled wing-hinge chordotonal organ represents the plesiomorphic condition, as our study now indicates, it appears that the evolution of the metathoracic ear is associated with a reduction in the number of chordotonal organ nerve cells. We propose two explanations for this phenomenon:

1. Cell reduction is non-adaptive. The reduction of cells is the result of certain non-adaptive constraints [i.e., historical, developmental, and architectural (Dumont and Robertson 1986)] that have imposed a restriction on the structure of the tympanal organ. Hasenfuss (1997), for example, suggests that there were difficulties with integrating all three cells into the evolving acoustical organ. This idea implies, however, that there was a necessary reduction in the overall size of the tympanal organ. Since other similar high-frequency ears in Lepidoptera (Geometroidea, Pyraloidea; see Cook and Scoble 1992; Scoble 1992) have tympanal organs that are larger, with four cells, it seems unlikely that the noctuoid ear could not ‘fit in’ all three cells. However, this remains a possibility.
2. Cell reduction is adaptive. While we acknowledge that adaptation is not the sole explanation for the structure of nervous systems, there may be advantages to possessing fewer cells in auditory systems such as that of the moth. Perhaps the reduction of cells from the three-celled condition reflects a sacrifice of a general sensitivity of the wing proprioceptor (covering a wider range of wing movement frequencies and amplitudes) for an increased speed and sensitivity to a specific kind of stimulus, the high frequency chirps of the bat echolocation call. The non-frequency discriminating properties of the noctuoid ear (Roeder and Treat 1957) would make additional cells required for this purpose redundant and the alerting function of the ear could be served better by fewer (and perhaps larger) cells. Possible analogies to the auditory system of the noctuoid ear are the relatively few cells which comprise the giant interneuronal network responsible for the startle reaction of various invertebrates (Roeder 1967), or the reduced number and increased diameter of sensory cells in the visual system of stalk-eyed flies (Diopsidae; Buschbeck and Hoy 1998).

In contrast to what we find in the Noctuoidea, there appears to be an increase in the number of cells from the pretympanal to tympanal condition in several other insect ears: the abdominal ear of grasshoppers (Acridoidea; Meier and Reichert 1990), the tibial ear of katydids (Tettigoniidae; Lakes-Harlan et al. 1991; Rössler 1992), the prothoracic ear of the tachinid fly (Tachinidae; Lakes-Harlan and Heller 1992; Robert et al. 1994; Edgecomb et al. 1995), and the tibial ear of the hissing cockroach (Blaberidae; Nelson 1980; Hoy 1992).

Each of these hearing organs is used primarily to identify and localize calling songs of either conspecifics or host species, in which case the insect may rely more on large numbers of cells to discriminate frequencies and/or to detect sounds at long distances. Interestingly, no significant differences in cell number were reported between the tympanal organ of the praying mantis and its proposed ancestral precursor in the cockroach (Yager and Scaffidi 1993). The mantid ear, like that of the moth, is used primarily for bat evasion (Yager et al. 1990). It would be worthwhile to compare the atympanate and tympanate conditions of abdominal ears in the Geometroidea and Pyraloidea (Lepidoptera), which also use their ears primarily for bat detection and avoidance.

It is difficult to interpret the evolutionary relationship between the tympanal organs of the Notodontidae and the other Noctuoidea, since the phylogenetic relationship between these taxa remains unclear. Although some have argued the ears of these groups evolved independently, most currently accepted phylogenies place the Notodontidae within the Noctuoidea lineage, rendering a separate evolutionary pathway for the metathoracic ears unlikely (for discussion, see Miller 1991). The unresolved origins of the Notodontidae within the Noctuoidea clade makes it currently impossible to determine whether or not there has been an evolutionary reduction of cell numbers (i.e., from a three-celled proprioceptive chordotonal organ, to two cells in the Noctuoidea, to one cell in the Notodontidae), but this would be the most parsimonious explanation.

In the Noctuoidea (except Notodontidae), A1 is the most sensitive of the two auditory cells and has been proposed to be responsible for initiating the ‘turning away’ response, while A2 is less sensitive and proposed to be responsible for alerting the moth to a close-range bat and for initiating the random flight and diving behaviors (Roeder 1974). However, the Notodontidae, with only one auditory neuron in each ear, appears to execute similar bimodal bat-avoidance behaviors (i.e., steering away or random flight and diving; Surlykke 1984). This suggests that either A2 is unimportant in eliciting bat-avoidance behaviours (i.e., the A2 is vestigial and without a function in avoidance) or that, in the Notodontidae, the nervous system processes sound differently, so that the one auditory cell has assumed the role of two cells.

We have demonstrated that the Noctuoidea tympanal organ derives from a proprioceptive wing-hinge chordotonal organ, which has been conserved throughout the Lepidoptera. Such neural ‘parsimony’ (Dethier 1963) is not an unusual feature of insect nervous systems (see Yack and Fullard 1990). It appears that the transition from the pretympanal to tympanal condition included modifications not only to the peripheral skeletal structures (for example, tracheal and cuticular), but also to the sensory organ itself (for example, cell number and strand length). These neural modifications have been important for transducing and relaying information about bat calls to the central nervous system. No doubt, modifications to the sensory cells have been accompanied by corresponding changes to central and motor neurons that govern the evasive flight maneuvers. Considering that the Noctuoidea tympanal organ and its atympanate homologue are easy to locate (due to their isolation from neighboring tissues within the body cavity) and structurally simple (having one to three cells that are easily characterized), we suggest that this system offers unique opportunities for studying changes to the nervous system that accompanied the evolution of hearing, and the behaviors associated with hearing, in insects.

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