Structure, Development, and Evolution of Insect Auditory Systems

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ABSTRACT This paper provides an overview of insect peripheral auditory systems focusing on tympanate ears (pressure detectors) and emphasizing research during the last 15 years. The theme throughout is the evolution of hearing in insects. Ears have appeared independently no fewer than 19 times in the class Insecta and are located on various thoracic and abdominal body segments, on legs, on wings, and on mouth parts. All have fundamentally similar structures—a tympanum backed by a tracheal sac and a tympanal chordotonal organ—though they vary widely in size, ancillary structures, and number of chordotonal sensilla. Novel ears have recently been discovered in praying mantids, two families of beetles, and two families of flies. The tachinid flies are especially notable because they use a previously unknown mechanism for sound localization. Developmental and comparative studies have identified the evolutionary precursors of the tympanal chordotonal organs in several insects; they are uniformly chordotonal proprioceptors. Tympanate species fall into clusters determined by which of the embryologically defined chordotonal organ groups in each body segment served as precursor for the tympanal organ. This suggests that the many appearances of hearing could arise from changes in a small number of developmental modules. The nature of those developmental changes that lead to a functional insect ear is not yet known.

INTRODUCTION

Hearing plays a crucial role in the lives of many insects: for some it provides a means to detect and evade predators, others use species specific acoustic signals to locate and select appropriate mates, and a few parasitoids home in on singing insects as hosts for their hungry offspring (Bailey, 1991). Although only a small proportion of all insect species can hear, the diversity of insect ears is astonishing: we know of at least 19 independent evolutions of audition among insects and that number is certain to grow. There is hardly a body part that does not, on some insect, sport an ear, and that includes wings, legs, and mouth parts.

The goal of this paper is to provide overview of insect peripheral auditory systems. It will be selective in two senses: (1) there will be a strong emphasis on discoveries and developments in the last 15 years. Details of earlier work are readily available in the excellent and comprehensive review by Michelsen and Larsen (1985). The mid-1980s also marked the beginning of a renaissance in the field of insect audition with the discovery of several “new” ears and, even more importantly, a shift in emphasis toward attempts to understand the evolution of insect auditory systems using developmental and comparative strategies; (2) this paper will deal only with pressure detection. The important topic of near-field sound reception (usually low frequency sound detected by socketed hairs) is well reviewed by Römer and Tautz (1992).

FUNDAMENTALS OF THE PRESSURE RECEIVER

Detection of the pressure component of sound allows sensitive detection of acoustic signals over long distances, whereas velocity and acceleration detection are limited to short distances and low frequencies (Römer and Tautz, 1992). Since the efficiency of signal production for small creatures like insects increases dramatically for frequencies greater than a few kilohertz (Michelsen, 1992), pressure-detection hearing makes particularly good sense for intraspecific communication. All animals, vertebrate and invertebrate, use a sensory organ of the same basic structure—the tympanate ear—for this purpose.

Functional Anatomy

The tympanate ear comprises three anatomical and functional parts: the tympanum, the tracheal sac, and the tympanal organ (shown in Fig. 1 using the noctuid moth ear as an example).

The tympanum is simply a region of very thin cuticle that is set into vibration by the oscillating difference in pressure between its two sides (Figs. 1, 3, 5). The typical insect tympanum is round or oval and membranous in appearance. Tympanal thickness in insects ranges from 1 µm in cicadas (Young and Hill, 1977) to 40–100 µm in wetas (the ensiferan family Stenopelmetidae; Ball and Field, 1981). There is often a distinct cuticular rim around the circumference that may help isolate it from body movements. Green lacewings (Neuroptera) have the smallest of known insect tympana with an area of 0.02 mm² (0.6 mm longest dimension; Miller, 1970); the area in cicadas (Homoptera) may

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reach 4 mm² (Young and Hill, 1977). The circular, uniform noctuid moth tympanum described below is one of the simplest among insects (Fig. 1). More complex tympana are non-homogeneous in thickness and sometimes have cuticular structures on the tympanum itself that affect vibration (some aquatic hemipterans, for instance, have a club-like appendage; Prager, 1976). The tympanum of locusts is among the most complex. It is divided into four regions, each associated with a group of sensilla in the tympanal organ (Müller’s organ). A complex interaction between the frequency-dependent tympanal vibration in each region and the separate biomechanical characteristics of Müller’s organ yields frequency discrimination based on a “place code” functionally analogous to that of the mammalian cochlea (Breckow and Sippel, 1985; Inglis and Oldfield, 1988; Stephen and Bennet-Clark, 1982).

The pressure changes of a sound wave will have little effect on a membrane unless the medium on the inner side of the membrane has a characteristic acoustic impedance (a function of density) matched to that of the external medium. For this reason, animals listening to airborne sound have an air space or air sac on the internal side of the tympanum; the mammalian middle ear is an example. For insects, this is an enlarged trachea or a tracheal sac tightly apposed to the inner surface of the tympanum (Figs. 1, 3, 5). There are rare exceptions: the green lacewing (on the radial vein of the wing; Miller, 1970) and some—but not all—aquatic hemipterans (on the mesothorax and metathorax; Arntz, 1975) have tympana backed by fluid.

The tympanum converts the pressure changes of a sound wave to an in-and-out motion of the membrane. This mechanical signal must be transduced to neural signals—the function of the tympanal organ. This is made up of chordotonal sensilla (also called scolopidia; Fig. 2) that do not appear to be substantially modified for sound reception as opposed to their mechanoreceptive functions throughout the insect body; they are classified as Type 1 sensilla (McIver, 1985). Field and Matheson (1998) provide extensive and excellent review of chordotonal organ anatomy and function. In tympanal chordotonal sensilla, a modified stereocilium with a 9*2+0 structure that originates in the dendrite of the single bipolar sensory neuron extends through a tubular space formed by the scolopale cell and inserts into the extracellular scolopale cap (Fig. 2B). The scolopale rods are intracellular structures composed of densely packed microtubules that form a sleeve around the cilium and also attach to the cap. An attachment cell surrounds the distal portion of the scolopale cell and the scolopale cap; it links the scolopidium to the vibrating membrane directly or through a ligament (for instance, Fig. 5C, D). In this way, the sensillum can be stretched by the differential movement between the tympanum and the less movable cell body with its surrounding glial cells. In a few cases (geometrid moths, cicadas, mantids; Kennel and Eggers, 1933; Michel, 1975; Yager and Hoy, 1987), some or all of the scolopidia in the tympanal organ have an inverse anatomy with the scolopales pointed away from the tympanum—the attachment cell anchors the sensillum to a relatively motionless structure and the opposite end is connected directly or indirectly to the tympanum. In terms of stretching the sensillum, this is mechanically equivalent to the more normal case, and the significance of these inversions is not known. The chordotonal sensilla of all tympanal organs studied to date are mononematic (the dendritic cilium attaches...
directly to a scolopale cap rather than a tube) and monodynal (one bipolar neuron per sensillum).

Tympanate insects vary widely in the number of scolopidia in the tympanal organ. There is only one chordotonal sensillum in the tympanal organ of notodontid moths (Surlykke, 1984) and sphinx moths (Göpfert and Wasserthal, 1999b). There are 1,300–1,500 in cicadas (Michel, 1975; Young and Hill, 1977) and almost 2,000 in the primitive pneumoridae grasshoppers (van Staaden and Römer, 1998). Many insect tympanal organs have 50–100 scolopidia. There is as yet no satisfactory explanation for this diversity. The number of sensilla does not correlate well with absolute sensitivity. In a case such as the pneumoridae (described in more detail below) where distance from the calling male affects the behavior of the female, the large number of scolopidia could provide intensity range fractionation for improved relative intensity measurement. Physiological evidence of intensity range fractionation comes from noctuid moths (see below) and some tettigoniids where several receptor cells tuned to the same frequency have substantially different thresholds (Kalmring et al., 1978). The analogous range fractionation for sound frequency occurs in the tympanal organs of ensiferans where there is a linear, tonotopic arrangement of sensilla (Oldfield, 1982; Oldfield et al., 1986; Stumpner, 1996), but Kalmring et al. (1990) did not find increased frequency range or resolution with increasing sensillum number among seven species of tettigoniid.

The necessary and sufficient stimulus for generating a receptor potential in the bipolar neuron of an auditory scolopidium is a longitudinal stretch of the cilium. While it seems evident that deformation of the cilium must lead to permeability changes that, in turn, generate a receptor potential (French, 1988), little is known about the actual mechanism. In other systems, it is clear that tension does not simply “stretch open” holes in the membrane to increase permeability; the response is due to the opening of specialized channels (French, 1992). These mechanically activated channels have been found throughout the animal kingdom, and their opening most often non-specifically increases the flow of monovalent cations (Hoger et al., 1997). However, they have not yet been unequivocally demonstrated in insect Type I sensilla.

The axons of the bipolar neurons terminate in the CNS in an auditory neuropile where they synapse with local and intersegmental auditory interneurons (reviewed in Michelsen and Larsen, 1985; Pollack, 1998). The afferent projections of all species studied to date are solely ipsilateral. In some insects, the auditory

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Fig. 2. A: Schematic drawing of three chordotonal sensilla in a tympanal organ. The somata of the bipolar sensory neurons (b) are surrounded by accessory cells (ac). The scolopale cell (sc) surrounds the dendrite of each neuron. Attachment cells (at) link the scolopidia with the tympanum (ty). B: Enlargement of the boxed region in A showing the relationship between the cilium of the neuron, the scolopale rods, and the scolopale cap. The locations of the two transverse sections are indicated by the dashed lines. Note that the cilium and the rods are all attached to the extracellular cap, and the cap is embedded in the attachment cell. Modified from Gray EG, 1960. The fine structure of the insect ear. Phil Trans R Soc B 243:75–94.
neuropile is entirely within the ganglion of the same body segment as the ear (crickets, tettigonoids), but more commonly, the afferents send branches into one or more adjacent ganglia (locust, cicada, mantis, tachinid fly). Where it has been identified, the region of the ganglion occupied by the auditory afferents is quite consistent across taxa (Ball and Field, 1981; Boyan et al., 1990; Esch et al., 1980; Halex et al., 1988; Römer et al., 1988; Wohlers et al., 1979; Yager and Hoy, 1987). The dominant and most consistent area is a dense neuropile just ventral to the ventral intermediate tract (VIT) known as the medial ventral association center (mVAC); also called the anterior intermediate sensory neuropile and the anterior ring tract, but these terms are not consistent with established CNS naming conventions; Field and Matheson, 1998; Pflüger et al., 1988).

This is an established mechanoreceptive processing region that receives chordotonal input in other insects (Hustert, 1978; Merritt and Whittington, 1995; Pflüger et al., 1988). Auditory terminations in mantids are unconventional (Yager and Hoy, 1987). In orthopterans, the afferents are grouped by their optimal frequency range; this division persists in mVAC in the form of a tonotopic organization (Halex et al., 1988; Römer, 1983; Römer et al., 1988; Stumpner, 1996; Wohlers and Huber, 1985).

**Capabilities**

Tympanate ears offer the advantages of good sensitivity at high frequencies and effective operation over a broad frequency range. Auditory thresholds for the most sensitive insects—pneumorid grasshoppers, for instance (van Staaden and Römer, 1998)—are in the range of 0–20 dB re: 20 μPa (=dB SPL), comparable to most mammals. For the majority of tympanate insects, best sensitivity is between 30 and 50 dB SPL, and there are some (nymphalid butterflies, some mantids) that are less sensitive (60–70 dB SPL). It is worth noting, however, that threshold comparisons across studies are difficult to interpret because of differences in measurement technique and threshold criteria. Michelsen and Larsen (1985) provide a less ambiguous summary from the literature: a vibrational displacement amplitude of 1 Å is sufficient to stimulate tympanic receptors of crickets, tettigonoids, locusts, and noctuid moths. Sphinxid moths are also at least this sensitive (Roeder, 1972).

For small animals like insects, the physics dictates that tympanate hearing below 1–2 kHz is rarely feasible (Michelsen, 1992), but many insect ears (crickets and cicadas, for instance) are tuned to match species-specific calling songs in the 2–6 kHz range. A number of insects—additionally or exclusively—have ultrasonic hearing for bat detection or for intraspecific communication or for both. Maximal sensitivity is most commonly at 30–60 kHz, the same frequency range most used by bats for their echolocation cries. Some pyralid moths (Spangler, 1987), tettigonoids (Morris et al., 1994), and mantids (Triblehorn and Yager, 2000) have their best hearing at frequencies ≥100 kHz. Not surprisingly, auditory system tuning for intraspecific communication tends to be much narrower than for predator evasion.

Virtually all hearing animals have two ears widely separated on the body, and this serves a vital behavioral function. By comparing the intensity or phase or time of arrival of sound at the two ears, an animal can determine the direction to a sound source; Michelsen (1998) provides a detailed review for insects. The pattern of two widely-separated ears holds for almost all insect auditory systems, but there are exceptions: praying mantids have a single ear in the ventral midline and lack directional hearing (Yager and Hoy, 1989); the auditory organs on the mouth parts of sphingid moths are near the midline and non-directional (Göpfert and Wasserthal, 1999a; Roeder, 1972); tachinid flies have two ears very close together on the anteroventral thorax, but achieve directionality through a novel bioacoustic mechanism (Miles et al., 1996; Fig. 7).

Ears on wings (green lacewings and two lepidopteran groups) pose a special problem since the ears are in constant motion during flight when hearing is behaviorally important. Green lacewings do not show evidence of auditory directionality (Miller, 1984).

**BRIEF OVERVIEW OF INSECT EARS**

**Taxonomic Distribution**

Tympanate hearing is scattered throughout the insect world with no obvious taxonomic pattern. Figure 3 shows the known distribution among orders and gives the minimum number of independent evolutions within each order. The pattern is patchy even at lower taxonomic levels in some otherwise tympanate lineages because of secondary loss (Gwynne, 1995; Otte, 1990; Yager, 1990).

**Locations on the Body**

At first impression, about the only place that insects seem not to have ears is on the sides of their head. Legs, mesothorax, metathorax, abdomen (various segments), wings, mouth parts are all home for ears in some group (Yack and Fullard, 1993). By far the most commonly eared body region is the caudal thorax/rostral abdomen (T2 – A2); this is the location of 12 of the 19 known peripheral auditory systems. There are several possible reasons for this; it is often the widest part of the body and hence provides greatest directional cues; the delicate tympana may be less vulnerable to damage; there are more and larger trachea and tracheal sacs available for impulse matching.

**Two Contrasting Examples**

**Noctuid Moths.** Anatomically, the noctuid ear is one of the simplest among insects. It is typical of moths in the superfamily Noctuoidea, which includes the noctuids, arctiids, notodontids, and allied families. The anatomy has been described by Eggers (1919), Richards (1933), Roeder and Treat (1957), and Ghiradella (1971) and reviewed by Spangler (1988a).

The two ears are positioned on the dorsolateral thorax at the junction with the abdomen (Fig. 1A). Each tympanum lies in a shallow cavity that in most cases has a movable covering or “hood.” They face posteriorly (more ventrally in arctiids). The “hood” is an extensively circular with a diameter in the range of 0.5–2 mm depending on the size of the moth. There is a rim of dense cuticle; a short projection (the “Bügel”) from the medial rim extends into the body (Fig. 1B). The tympanal membrane is typically 0.5–1.0 μm thick and quite delicate. Behind the tympanum is a large tracheal sac that increases sensitivity (by impedance matching), but
The tympanal organ comprises two chordotonal sensilla of typical structure (but only one in notodontids; Surlykke, 1984) fixed by attachment cells directly to the center of the tympanum (Fig. 1B; Ghirardella, 1971; Yack and Roots, 1992). The two afferent neurons, the A1 and A2 cells, have the same broad tuning to 25–50 kHz, but some branches of A2 terminate more dorsally (Boyan et al., 1990). There are at least ten bilateral pairs of interneurons originating in the metathorax and mesothorax that receive strong auditory input, and most send their axons to the brain (Boyan and Fullard, 1986, 1988; Boyan and Miller, 1991; Boyan et al., 1990).

Despite the relative simplicity of the auditory system, noctuids are capable of performing evasive maneuvers in response to bat echolocation cries that are appropriate in direction and behavioral intensity to the location of the approaching bat (reviewed in: Fullard, 1996; Roeder, 1967). The intensity range fractionation provided by the A1 and A2 cells underlies a switch in type of escape behavior as the distance between bat and moth decreases. The maneuvers increase the probability of survival by 40–50%. Some species of noctuid moths have additionally (and secondarily) incorporated acoustic signaling into their courtship (for instance: Conner, 1987; Sanderford and Conner, 1990).

Cricket and Bushcrickets. The ears of the orthopteran suborder Ensifera (crickets, bushcrickets, weta, and allied families) differ in several anatomically and functionally important ways from the simple ears of moths (Bailey, 1990; Ball and Field, 1981; Ball et al., 1989; Mason, 1991; Michel, 1974). Most obvious is location: ensiferan ears are on the proximal tibiae of the prothoracic legs (Fig. 4A). Crickets and bushcrickets have two tympana on each tibia. In the former, they are exposed on the anterior and posterior surface; the anterior tympanum plays little, if any, role in sound reception. Bushcrickets also have an anterior and posterior tympanum on the tibia (both of which contribute to sound reception), but they are most often in slits or chambers. The diversity of these structures associated with the tympana in tettigoniids is remarkable, and their effects on hearing have been a source of extended debate. Some data (reviewed in Bailey, 1990) suggest that they confer heightened sound localization favoring the direction of the slit openings, and that, at least in some cases, the slits/cavities may play an important role in tuning the auditory response. However, other authors (Lewis, 1974; reviewed in Michelsen, 1992; 1998) have pointed out that even for larger bushcrickets calling at high frequencies, the sound wavelengths are long compared to the dimensions of the slits and chambers. In this situation, the bioacoustics predict a negligible effect of the accessory structures on tympanal vibration, and measurements have shown minimal diffraction of sound by the leg (Michelsen et al., 1994a).

The posterior tympanum of a medium-size cricket like *Teleogryllus commodus* is oval, with dimensions of approximately 0.8 × 0.3 mm; the tympanal thickness is 2–4 µm (Young and Ball, 1974). The vibration characteristics of the tympanum—especially the role played by the tympanum in tuning the auditory system—have been controversial. Recent studies on crickets (Larsen et al., 1989; Popov et al., 1994) and bushcrickets (Bangert et al., 1998) have found that the tympanum is not strongly tuned to the calling song, which suggests a secondary source of tuning in the mechanics of the vibration of trachea or associated tissue or in the mechanics of the receptor cells themselves.

A large tracheal expansion is apposed to the inner surface of the tympanum (Fig. 4B,C). The tympanal...
tracheal sac is part of a tracheal system that is open to the exterior through the enlarged first thoracic spiracle and that communicates with the spiracle and tympanal air sac on the contralateral side. In contrast to moths, the tracheal system plays a crucial role in sound reception beyond simple impedance matching by providing access for sound to the inner surface of the tympanum. The force moving the tympanum is the net pressure resulting from sound striking the inner and outer surface, which, in turn, is a complex function of sound frequency along with the geometry and acoustics of the tracheal system (a “pressure difference” receiver; Michelsen, 1998). In crickets, tympanal motion is determined by both the sound striking the external surface and the sound reaching the internal surface through the ipsilateral and contralateral spiracles (Michelsen et al., 1994b). The internal routes introduce phase delays that vary with direction; at the calling song frequency the contribution from the ipsilateral spiracle is about three times larger than that from the contralateral spiracle, but the latter is crucial for directional hearing.

The tympanal organ in these insects (the “crista acustica”) is not attached directly to the tympanum, but to the wall of the tympanal tracheal sac (Fig. 4B,C). It is a linear array of 60–70 chordotonal sensilla (20–50 in bushcrickets) oriented along the longitudinal axis of the tibia. Intracellular recordings from individual bipolar sensory neurons at different locations along the array have demonstrated a tonotopic organization: distal sensilla respond to higher frequencies and proximal sensilla to lower frequencies (Oldfield, 1982; Oldfield et al., 1986; Stumpner, 1996). The number of sensilla devoted to each portion of the overall frequency range varies with species (Kalmring et al., 1990; Oldfield, 1984, 1988). Typically, the receptors cluster in three frequency ranges: the greatest number are tuned
around the calling song frequency (3–5 kHz for most crickets), and the smallest number have best frequencies over 19 kHz (Imaizumi and Pollack, 1999).

The auditory afferents travel to the prothoracic ganglion in the leg nerve (Nerve 5); they form a dense auditory neuropile in mVAC. The tonotopy that originates with the receptors is preserved in the organization of the auditory neuropile (Oldfield, 1983; Römer, 1983; Römer et al., 1988; Stumpner, 1996). There are at least seven bilateral pairs of auditory interneurons, and their physiology and role in processing auditory information have been studied extensively (reviewed in: Pollack, 1998; Schildberger et al., 1989).

The fossil record (Carpenter, 1992) and comparative studies (Gwynne, 1995) suggest that the earliest—and still dominant—function of hearing in the Ensifera was intraspecific communication. After the appearance of echolocating bats 65–85 million years ago (Novacek, 1985), many lineages added bat evasion based on ultrasonic hearing (Libersat and Hoy, 1991; Mason et al., 1998; Nolen and Hoy, 1986), although there are many instances of secondary loss of this function accompanying brachyptery and flightlessness (Otto, 1990).

**RECENT DEVELOPMENTS**

**Newly Discovered Ears**

**Dictyoptera**

Praying mantids (suborder Mantodea). Praying mantids are highly visual, diurnal predators, and their huge eyes largely defined our perception of this insect’s sensory world. That was a limited view since mantids also have sensitive hearing (Yager and Hoy, 1986, 1987; reviewed in Yager, 1999). They are the only known terrestrial animals with a single ear. Not all mantids hear, however, and even among the eared there are several morphological and functional variations (Triblehorn and Yager, 2000; Yager, 1989, 1990, 1996a). The description that follows applies to the form described above. If they can hear at all, the females with shortened wings and the loss of flight ability. In reduced hearing in females is uniformly associated with the poorly developed metathoracic groove with tiny tympana and greatly reduced tympanal tracheal sacs. If they can hear at all, the females of mantids there is no appreciable sensitivity below 10 kHz. The lowest thresholds we have encountered were 20–30 dB SPL, although sensitivity for most species is in the 40–60 dB SPL range and a few are less sensitive at 65–75 dB SPL (Triblehorn and Yager, 2000; Yager, unpublished observations). There is no physiological or behavioral evidence to suggest that mantids can discriminate different frequencies (however, see below concerning a two-eared mantis).

Mantids are unusual in that over 30% of the genera show strong sexual dimorphism in the structure and function of the auditory system (Yager, 1990). In these cases, males have sensitive hearing and the ear anatomy described above. If they can hear at all, the females have auditory thresholds >90 dB SPL. They have a poorly developed metathoracic groove with tiny tympana and greatly reduced tympanal tracheal sacs. Reduced hearing in females is uniformly associated with shortened wings and the loss of flight ability. In the few species where males have short wings, they too have lost their auditory sensitivity.

Ultrasound triggers a complex, multi-component behavioral response after 50–100 ms in flying mantids. The aerodynamic result is a steep, spiral power dive. In both flight room and field studies, the response to
ultrasound successfully prevented capture by echolocating bats (Triblehorn, unpublished observations; Yager and May, 1990; Yager et al., 1990).

Cockroaches (suborder Blattodea). Neurophysiological recording from a broad taxonomic sampling of cockroaches (14 species from 4 of the 5 families) found no evidence of hearing above 5 kHz or of a midline ear as in mantids (Yager and Scaffidi, 1993). However, Shaw (1994a,b) has shown that *Periplaneta americana* can detect airborne sound below 5 kHz using the subgenual organs (lowest mean threshold of 55 dB SPL at 1.8 kHz; threshold rises steeply above 2 kHz). It has long been known that the sensitivity of these tibial chordotonal organs to mechanical vibration is exceptionally high (reviewed in Shaw, 1994a). However, cockroaches have no tympana on their legs, so a major question is how the airborne sound can be coupled effectively to the subgenual organs. Based on data using isolated leg preparations, Shaw (1994b) suggested that the sound route is through the tracheal system of the leg rather than directly through the external cuticle or indirectly through ground vibra-

Fig. 5. **A:** The single ear of praying mantids is located in a deep groove (g) in the ventral midline between the metathoracic coxae (mtc) as also indicated by the arrow in the upper drawing. In one small lineage, a second, serially homologous midline ear has evolved between the mesothoracic coxae (msc) that is tuned to 2–4 kHz rather than ultrasound and operates independently of the metathoracic ear. Scale bar = 1 mm. **B:** Transverse section through the metathoracic ear. The tympana (ty) form the walls of the deep groove and are backed by tracheal sacs (ts). Distinctive cuticular knobs (k) mark the anterior end of the groove. Scale bar = 100 μm. **C:** A horizontal section through the metathoracic ear; only the anterior-most portion is shown. The tympanal organ (to) of 35–45 scolopidia attaches to the tympanum at its extreme anterior end and is surrounded by haemolymph. Scale bar = 100 μm. **D:** An enlargement of the medial end of the tympanal organ showing three scolopales (s; rods and cap). The elongated nuclei of the attachment cells (at) are visible in the lower left (toward the tympanum); the nuclei at upper right are those of scolopale cells (sc) and accessory cells. The attachment cells are associated with a long ligament that actually connects the tympanal organ to the tympanum. Scale bar: 10 μm. A is reprinted from Yager DD. 1996a. Serially homologous ears perform frequency range fractionation in the praying mantis, *Creobroter* (Mantodea, Hymenopodidae). J Comp Physiol A. 178:463–475, with permission.
tions. The subgenual organs of all six legs show similar responsiveness.

No behavioral function for *P. americana’s* sensitivity to airborne sound has yet been discovered. However, sound production in defensive contexts is well known among cockroaches, and acoustic signals—probably detected by the subgenual organs—play an important role in social interactions in two genera, *Nauphoeta* and *Gromphadorhina*, in one of the other cockroach families (Hartman and Roth, 1967; Nelson, 1980; Nelson and Fraser, 1980).

**Coleoptera**

Tiger beetles (*Cicindelidae*). Spangler (1988b) uncovered the first evidence of hearing in this huge (350,000 species) insect order during field studies, and he confirmed that *Cicindela lemniscata* has sensitive ultrasonic hearing using a behavioral assay. A recent comparative study (Yager et al., 2000) has shown that hearing is widespread in the very large, worldwide genus *Cicindela*, but it is not universal in the family *Cicindelidae*.

Ablation experiments localized two tympana to the enlarged dorsal portion (tergum) of the first abdominal segment (Fig. 6A). In species with low threshold hearing like *C. lemniscata* and *C. marutha*, the tympana are thin and almost completely transparent except for their medial portions. Selective ablations while recording auditory responses neurophysiologically (Yager and Spangler, 1995) showed that damage to a small triangular region 0.3–0.5 mm long in the anterolateral corner of the tympanum raised thresholds by >25 dB, but even extensive destruction of other areas had only small effects. The cuticle in the anterolateral corner is floppy and membranous as opposed to the stiff cuticle of the rest of the tympanum. Scanning electron microscopy shows it to be finely ridged with a central invagination or “dimple.” The vibration mechanics of the tiger beetle tympana are not known.

Beneath each tympanum is a large tracheal air space that is part of an extensive series of air sacs in the abdomen and thorax. The tympanal air sac opens externally through a greatly enlarged first abdominal spiracle on the lateral body wall just below the tympanum.

The tympanal afferents travel in a branch (nsa1a) of the dorsal root of the first abdominal ganglion. The only nerve obviously associated with the tympanum is very small branch that can be traced to the region just under the anterolateral corner near the central invagination. The tympanal organ itself has not been identified, but hook electrode recordings from nsa1a suggest 4–20 auditory afferents (hence 4–20 scolopidia).

The auditory system of *C. marutha* is quite sharply tuned to 30–35 kHz with lowest thresholds of 50–55 dB SPL (Yager and Spangler, 1995). Many tiger beetles occasionally produce stridulatory sounds at 30–35 kHz when on the ground, but there is no behavioral evidence of intraspecific communication (Pearson, 1988). In fact, the ears are fully covered by the wings and elytra except in flight, so the auditory sensitivity of potential receivers on the ground may be very low. Ultrasound does, however, trigger a complex multi-part behavior.
with a latency of <100 ms in flying beetles (Yager and Spangler, 1997). A major component is the rapid and powerful swing of the non-flapping elytra back into the flapping hindwings. This deforms the hindwings—presumably altering the aerodynamics—and at the same time creates trains of loud ultrasonic clicks. Arctiid moths, using a different mechanism, produce acoustically similar click trains that effectively deter or disrupt bat attacks (Acharya and Fenton, 1992; Dunning and Roeder, 1965).

**Scarab beetles (Scarabidae).** Behavioral observations in the field (Forrest et al., 1995) also led to the discovery of paired hearing organs in one of the largest beetle families, the Scarabidae (Forrest et al., 1997). The currently known distribution of these ears is limited to two tribes of one scarab subfamily, the Dynastinae. Both phylogeny and anatomy make it clear that hearing evolved independently in the Cicindelidae and Scarabidae.

The ear of the scarab *Euetheola humilis* follows the standard structural design for tympanate ears, but its location is novel (Fig. 6B,C; Forrest et al., 1997). The tympana are located dorsolaterally in the neck membranes just under the pronotal shield. The roughly oval tympana are 1–2 mm in long dimension and 2–3 μm thick. The structural contrast between the scarab tympanum and the surrounding tissue—cervical membrane that is 5 μm thick—is substantially less than in other insects. A tracheal sac underlies each tympanum. The location of the tympana offers these beetles the possibility of controlling auditory sensitivity behaviorally. While walking or flying, the neck is extended, the head is away from the pronotum, and the tympana are exposed. When the head is retracted back to the pronotum, the tympana are covered and auditory responsiveness disappears (Forrest et al., 1997).

The tympanal organ of *E. humilis* comprises 4–8 chordotonal sensilla and attaches to the tympanum by accessory (attachment) cells (Forrest et al., 1997). The attachment site is not at the center, but at the dorsomedial apex of the membrane. Both neurophysiological and behavioral audiograms show that *E. humilis* is most sensitive to frequencies of 40–50 kHz with lowest thresholds of 55–60 dB (Forrest et al., 1995, 1997). Ultrasound triggers a stereotyped, lateralized response with a very short latency (30–60 ms); a major component of the response is a head roll. Although there is strong indirect evidence that hearing helps scarabs evade echolocating bats as it does in several other insects, their auditory behavior is unique because it also occurs during walking. In all of the other insects studies so far, complex, short-latency evasive responses to ultrasound occur only when the animal is flying. The response of *E. humilis* while walking must afford protection from dangers other than bats, since their auditory sensitivity is not high enough to detect the approach of gleaning bats (Faure et al., 1993).

**Diptera.** Based on behavior, hearing had been suspected in some parasitoid flies since the mid-1970s (Cade, 1975; Soper et al., 1976), but no one could have anticipated just how unusual the fly ear would prove to be when it was first described by Lakes-Harlan and Heller (1992) and Robert et al. (1992). Similar, but independently evolved, ears are found in parasitoid flies in two dipteran families, Tachinidae and Sarcophagidae; most thoroughly studied are the orminine tachinids, *Orminia ochracea* (Robert et al., 1994, 1996a) and *Therobia leonidei* (Lakes-Harlan and Heller, 1992).

The two tachinid ears are adjacent and essentially fused in the ventral midline at the rostral end of the prothorax, just behind the head (Fig. 7). A single large airspace spanning both ears provides impedance matching for the two tympana. (Superficially, the ear looks like a bubble under the fly’s “chin.”) The ears are sexually dimorphic, especially in size: the total width of the two ears is 1.7 mm in females and 1.1 mm in males. The attachment points of the two chordotonal organs are separated by only 0.5–0.7 mm.

The female tympana are very complex, roughly triangular membranes that are about 1 μm thick (Fig. 7A). They are radially corrugated with much finer corrugations centrally than peripherally. A cuticular bar (part of the intertympanal bridge) extends from the midline toward the center of the tympanum. There is a small invagination (tympanal pit) near the end of each bar. The tympanal organ (“bulba acustica”) is attached anteriorly to the tympanum at the tympanal pit by way of a thin, stiff apodeme and posteriorly by a ligament to the wall of the tracheal space. Thus, both tympanal organs span the rostral-caudal extent of the same tracheal space. Each of the ellipsoidal tympanal organs contains longitudinally-oriented chordotonal sensilla of conventional structure. Although the ear anatomies of *T. leonidei* and *O. ochracea* are largely the same, the former has at least 200 sensilla in each bulbula acustica as opposed to only 70 in the latter. The axons of the bipolar sensory neurons join the frontal nerve and enter the thoracic ganglionic complex where they have ipsilateral, medial terminations in all three thoracic neuromeres. The exact location of the auditory afferent terminal fields in relation to the ganglionic tracts and neuropiles is not as yet known. Stumpner and Lakes-Harlan (1996) have identified six bilaterally symmetrical pairs of auditory interneurons in *T. leonidei*, at least three of which carry information to the brain.

Mantids and flies both have midline ears, but, nonetheless, have fundamentally different peripheral auditory designs. The mantis has a single ear in which two tympana function together bioacoustically and two tympanal organs pool their information in the CNS to create a single “message” ascending to the brain. There is a gain in sensitivity at the expense of directionality. Despite their intimate adjacency, the two ears of the tachinid fly nonetheless function separately and are thus able to provide directional information. This should not be possible given any of the known mechanisms for directional hearing (Michelsen, 1998), and, in fact, these small insects use a unique and previously unknown mechanism.

Miles et al. (1995) and Robert et al. (1996b, 1998) have used laser vibrometry, experimental manipulation, and mechanical modeling to discover and describe the directional mechanism (Fig. 7C). The key feature is mechanical coupling between the two tympana. The intertympanal bridge comprises the two cuticular bars—with the tympanal organ attachments at their ends—connected at a midline pivot point. The bridge links together the motion of the two tympana in the manner
of a flexible lever with a spring-like restoring force acting at each end. If the intertympanal bridge were a single rigid bar, an inward motion of the tympanum on one side would cause a simultaneous outward motion of equal amplitude on the other. However, the bridge actually comprises two bars with a non-rigid linkage that introduces damping and a time delay to the contralateral motion. The interaural intensity difference for a 5 kHz lateral sound source is 0 dB and the interaural time delay is 1–2 µs, neither of which are detectable by insect neural systems. As a result of the intertympanal coupling, tympanal vibration contralateral to the sound source is delayed by 50–60 µs and has 10–15 dB lower amplitude than the ipsilateral vibration. Recordings from the auditory afferents reveal a further delay amplification (the source is not clear) so

Fig. 7.  A: Scanning electron micrograph of the prothoracic ear of the tachinid fly Ormia ochracea; the head has been removed. The white arrowheads indicate the boundaries of the tympanum. The tympanal pit (tp) is evident at the end of each half of the intertympanal bridge. Two levels of striations radiate from the end of the bridge: medially the striations are very fine and laterally they are more widely spaced. Scale bar = 200 µm. B: A generalized tachinid fly (wings and distal legs removed) indicating the location of the ears beneath the fly’s “chin.” C: A schematic representation of the mechanical model to explain the novel mechanism for directional hearing in ormiine tachinid flies. Force and subsequent movement at the tympanal pits (arrows) causes the intertympanal bridge (long rectangles) to move around its fulcrum (F) in the midline. The diamonds (D) represent a combination of restoring force and delay. The connection between the tympanal pits and anchor points (A) is the tympanal organ and its ligaments. The lower drawing shows the relative motion of the two halves of the intertympanal bridge in response to sound coming from the left. Contralateral movement is in the opposite direction and has a lower amplitude than ipsilateral movement; it is also delayed by 50–60 µs (not shown). See text for further explanation. A modified from Robert et al. (1994); B modified from Colless and McAlpine (1991); C redrawn from Robert and Hoy (1998).
that the ipsilateral response leads the contralateral by approximately 320 µs, a delay long enough to be used by the CNS for directional processing. Miles et al. (1995) developed a mathematical model of the mecha

Female ormine tachinid flies orient accurately to a sound source and use this capability to locate hosts for their offspring. For O. ochracea, these are male field crickets (Robert et al., 1992); the European T. leonidei seeks out male bushcrickets (Lakes-Harlan and Heller, 1992). The optimal frequencies for tympanal vibration match the dominant calling song frequencies of the hosts: 5 kHz for O. ochracea and 30–40 kHz for T. leonidei. The parasitoid sarcophagids have not been studied in detail, but also appear to orient preferentially toward the dominant calling song frequencies of their hosts (cicadas; Soper et al., 1976). Once the female locates a singing male, she deposits first instar larvae on or near the host. The larvae burrow into the male where they feed and grow. Parasitization rates in a host population can be over 50% of the males (Lakes-Harlan and Heller, 1992; females are never hosts). Male ormine flies have smaller ears than females. The functional consequence for O. ochracea is a lower auditory sensitivity by 40–50 dB and tuning to >10 kHz. The behavioral role of hearing in males is not known.

Some Tympanate Groups Revisited

Many notable studies since 1985 have extended our knowledge of auditory anatomy, physiology and behavior in the “traditional” tympanate insects systems. These are discussed in a recent review volume (Hoy et al., 1998). I have selected three of the newest examples that have particular relevance to a discussion of insect ear evolution. 

Multi-Eared Grasshoppers. The South African pneumorid grasshopper Bullacris membracioides has an acoustic communication system in which males produce loud calls that are answered by the approaching females (van Staaden and Römer, 1997). After a characteristic acoustic duet, the male moves toward the female and mating ensues. Females can detect the male call at distances up to 2 km because the male call is loud (98 dB SPL at 1 m) and because female hearing is among the most sensitive of all insects (van Staaden and Römer, 1997). Thus, it is somewhat surprising to discover that these insects lack differentiated tympana, and even more surprising to find that they have six serially homologous pairs of functional auditory organs in their abdomen (van Staaden and Römer, 1998).

The “ear” in the first abdominal segment (A1) is located in the lateral (pleural) region and is clearly homologous with the more conventional ears of other locusts and grasshoppers (Meier and Reichert, 1990). The number of chordotonal sensilla in the tympanal organ is huge—almost 2,000; other chaeliferans have 70–100. The tympanal organ is attached to the lateral body wall by two unusually long bundles of attachment cells, each associated with its own group of scolopidia. Physiological recording from the afferents show lowest thresholds of 10–15 dB SPL at a best frequency of 4 kHz (mismatched to the dominant frequency of the male’s call, 1.7 kHz).

The next five abdominal segments (A2–A6) have serially homologous auditory organs. Although structurally similar to the A1 ear, these are smaller with only 11 sensilla each. They are tuned to 1.5–2.0 kHz, and the sensitivity decreases systematically in progressively caudal segments (thresholds of 58 dB SPL in A2 to 77 dB SPL in A6).

Strategic ablations combined with playback experiments to assess behavior have demonstrated that the physiological intensity range fractionation afforded by the six pairs of auditory organs has its counterpart in a graded series of behavioral responses that are important for successful reproductive encounters (van Staaden and Römer, 1998).

Ears on Wings. Although the tiny tympanate ears on the radial vein of green lacewing (Neuroptera) forewings have been studied in some detail (reviewed in Miller, 1984), the wing ears of Lepidoptera have not received equivalent attention. Early anatomical studies (Bourgogne, 1951; Vogel, 1912) identified “Vogel’s organ,” an ear-like structure with a chordotonal organ (12–40 scolopidia) at the base of the wings in some nymphalid butterflies (a family within the superfamily Papilionoidea, the “true butterflies”; Scoble, 1995). Using neural recording and a startle behavior, Swihart (1967) found evidence for functional auditory organs in two nymphalid subfamilies, Heliconiinae and Limenitidinae. Ear structure was similar in both subfamilies as was the sensitivity (60–65 dB SPL at the best frequency, 1.2 kHz). However, the heliconiine ears were localized to the hindwings, whereas ears in the Limenitidinae are on the forewings. Ribaric and Gogala (1996) have confirmed hearing in a third nymphalid subfamily (the Satyrinae; ears on forewings). A number of limenitidine species produce loud clicks during social interactions (Swihart, 1967).

Alar ears have recently been discovered in a second butterfly superfamily, the Hedyloidea. The initial tentative identification based on gross anatomy (Scoble, 1986) has now been confirmed and refined with histological and behavioral studies (Yack, personal communication). The ears are at the base of the forewings and appear the be built of structures homologous to Vogel’s organ in nymphalids. Hedylid butterflies are nocturnal, and they respond in flight to ultrasound with typical evasive behaviors.

The evolutionary relationships of the various butterfly wing-ears is a puzzle, in part because the phylogeny is unsettled. Parsimony suggests at least two independent evolutions, and there is ample precedent for repeated appearance of ears at the same body location in a lineage (see below). However, some anatomical features point to a common origin for alar ears in the two butterfly superfamilies (Yack, personal communication).

Ears on Mouth Parts. Roeder and colleagues (Roeder et al., 1970; Roeder, 1972; Roeder and Treat, 1970) found a unique auditory organ sensitive to ultrasound built into the mouth parts of species in one tribe of hawkmoths (Choerocampini, Macroglossinae, Sphinxidae; Fig. 8A). Sensitivity was 40–45 dB SPL at 25–35 kHz, and audition was presumed to aid bat evasion. Göpfert and Wasserthal (1999a,b) have extended the story by providing new behavioral and physiological information on choerocampine hearing and have also
added a fascinating anatomical and evolutionary twist with the discovery of mouth-ears in the Death's Head moth (Acherontia atropos) and some of its relatives, members of a different hawkmoth lineage (Acherontini, Sphinginae, Sphingidae).

In both sphingid groups, the two ears are located near the midline on each side of the proboscis (Fig. 8B,C). They have two disjoint parts: a more lateral labial palp that is specialized to increase sensitivity to airborne sound, and a medial labral pilifer modified for efficient transduction. In the choerocampines, the second segment of the palp is like a balloon. It is enlarged (1.0–1.5 mm across), filled with air, and has thinned cuticle, especially its medial wall, which is also devoid of scales (Fig. 8B). The pilifer has two lobes; the distal lobe is normally in close contact with the smooth medial surface of the palp. The pilifer is 0.2–0.3 mm long. Laser vibrometry shows that the medial face of the palp is the tympanum; other areas of the palp do not vibrate significantly in response to sound. The pilifer is a bit like the tonearm on a record player: the distal tip touches the tympanum and transmits the vibration to a single chordotonal sensillum located at the base of the pilifer.

The auditory organ is quite different in acherontine hawkmoths (Fig. 8C). The palp is not enlarged and does not have thinned cuticle. However, its medial face is deeply concave, and a tuft of broadened scales forms a “plate” protruding into the concavity. The scale plate’s surface area is approximately 1 mm². The pilifer has a single lobe, it is elongated (0.6–0.8 mm), and it is hinged at its base. In contrast to the choerocampines, the scale plate rather than the medial wall of the palp vibrates in response to airborne sound. Thus, there is no tympanum in any conventional sense. The pilifer has broad contact with the scale plate and rocks dorsoventrally around the hinge when the scale plate vibrates; this stimulates the single scolopidium at the base of the pilifer.

Both acherontine and choerocampine hawkmoths are sensitive only to ultrasound, generally at 20–40 kHz although a few species are tuned to 50–70 kHz. Göpfert and Wässerthal (1999a,b) found physiological thresholds of 50–55 dB SPL and behavioral thresholds 10–15

Fig. 8. A: The ears of choerocampiine and acherontiine hawkmoths are located in the mouth region (arrow). B: Dorsal view of the head of a choerocampiine hawkmoth. The labral palps lie just lateral to the proboscis (pr) and medial to the labial palps (pa); the palps are medial to the compound eyes (e). The left palp is moved laterally out of its normal position. The right palp is drawn in horizontal section showing that the interior is primarily air space. In their normal positions, the distal tip of the pilifer just touches the medial face of the palp, i.e., the tympanum. C: Frontal scanning electron micrographs of an acherontiine hawkmoth, Acherontia atropos. Left: Left palp is deflected laterally to expose the pilifer. Right: Dorsal close-up view of the left palp-pilifer in their normal positions. The pilifer contacts the dorsal surface of the palpal scale plate (sp) and moved dorsoventrally when the scale plate vibrates. The hinge is visible as a crease at the base of the pilifer. Scale bars = 1 mm. A modified from O’Toole C, editor. 1986. The encyclopedia of insects. New York: Facts On File Publications. 152 p; B redrawn from Roeder et al, 1970. C modified from Göpfert MC, Wässerthal LT. 1999a. Hearing with the mouth-parts: behavioral responses and the structural basis of ultrasound perception in acherontiine hawkmoths. J Exp Biol 202:909–918.
dB higher. They identified several ultrasound-triggered behaviors including some during tethered flight that are consistent with bat evasion.

Even though the ears are built of homologous parts, the phylogeny, the divergent anatomy, and the different sound-transducing devices all point to independent evolutions of hearing in the choerocampine and acherontine hawkmoths.

**EVOLUTION OF INSECT EARS**

In no other animal class has an exteroceptive sense with specialized peripheral organs evolved independently as many times as hearing has in the Insecta. The number of known independent innovations of insect ears is currently at 19 and climbing. By virtue of this diversity, insect audition provides a unique opportunity to study the evolution of new sensory systems. In other words, how does an animal go from earless to eared? Are there particular peripheral precursors present in all insects? What determines where on the body the ears appear? What genetic or epigenetic processes govern the transformation? What changes must occur in the CNS to process input from the new sensory modality? How do acoustically-triggered adaptive behaviors arise?

Ultimately evolution comes about by changes in developmental processes or their timing. Therefore, it makes sense to consider auditory development in the discussion of insect ear evolution. Boyan (1998) provides an extensive review of insect auditory system development.

**Why?**

Superficially, this question of ultimate causation has straightforward answers. Since we know the current functions of hearing in insects, we infer that the selective forces leading to hearing in different groups were the need to avoid predators, the need to attract and select appropriate mates, and the need of parasitoids to find hosts for their offspring (Bailey, 1991). The problem is that current function need not be the same as the original function.

Except for a few orthopterans (Carpenter, 1992), the fossil record of ears is very scant and provides little help. Comparative studies of both anatomy and acoustic behavior have, however, provided some strong clues. For instance, some moths use intraspecific acoustic communication in courtship, but it is widely accepted that this is a recent adoption of ultrasonic hearing and sound production originally in place as defense mechanisms against bat predation (Fullard, 1998; Sanderford and Conner, 1990). Comparative and neuroanatomical studies yield the same conclusion for locusts and grasshoppers (Otte, 1977; Reide et al., 1990). For the ensiferans, however, the historical pattern is probably reversed. Both fossil tegmina that appear highly specialized for sound production and legs with tibial ears are known that predate the appearance of echolocating bats (65–85 million years ago; Novacek, 1985) by at least 100 million years (Carpenter, 1992). Thus, ultrasonic hearing for defense seems to be a recent additional function to an ancient acoustic intraspecific communication system (Hoy, 1992).

The growing comparative literature on insect ears suggests that secondary loss of the auditory system is a common and important evolutionary pattern among tympanate insects. There are examples among katydids (Gwynne, 1995), moths (Scoble, 1995), grasshoppers (Mason, 1968), and crickets (Gwynne, 1995; Otte, 1990). It has probably occurred five times among the North American tiger beetles of the genus Cicindela (Yager et al., 2000), and no fewer the 14 times among the praying mantids (Yager, 1990). It is often, but not always, associated with auditory sexual dimorphism in which males hear and the females do not. There is also an extremely tight correlation of secondary ear loss with the secondary loss of flight (wing reduction or absence) in species that use hearing to evade bats (the exceptions are insects like crickets that also use acoustic intraspecific communication; Otte, 1990). The “Why?” in sexually dimorphic cases has an appealingly plausible answer: studies in several insect groups show that under certain ecological conditions, females can profitably “trade” flight for increased fecundity (Roff, 1986; Wagner and Liebherr, 1992; Zera et al., 1997). Flightless animals face no pressure from echolocating bats—the auditory system becomes a needless expense and disappears.

**When?**

In terms of absolute time, the absence of fossil ears poses the same problems noted above; Carpenter (1992) provides the best review. Specialized stridulatory structures were widespread in the Orthoptera by the Triassic, and tibial ears are present in some Haglidae (an ensiferan) from that period. Tympana on the first abdominal segment of Eocene locusts are known, but there is molecular and comparative evidence suggesting that pneumodir stridulation (and ears?) dates to the Jurassic (van Staaden and Römer, 1998). Cicadas had specialized tymbals by the Paleocene.

It is easier to deal with phylogenetic age, which can often be inferred from the results of comparative studies superimposed on hypothesized phylogenetic trees. For instance, it is clear that hearing appeared very early in the history of both of the two major orthopteran suborders (although after they had diverged, of course; Gwynne, 1995; Otte, 1977; Reide, 1987). This is also the case for the metathoracic mantis ear (Yager, 1989, 1990; however, the mesothoracic ear of hymenopodid mantids appeared very late; Yager, 1996a). At the other extreme, tachinid and sarcophagid fly ears are phylogenetically very recent innovations in their respective lineages (Edgecombe et al., 1995).

**How?**

What are the proximate mechanisms that bring about the transition from earless to eared? How is the normal pattern of development changed to yield an auditory organ composed of several tissue types organized into a structure that transduces airborne sound first into mechanical vibration and then into neural signals?

**Precursors.** Two lines of evidence have led to a growing consensus that all known insect tympanal organs derive from chordotonal mechanoreceptors (Boyan, 1993; Edgecomb et al., 1995; Lewis and Fullard, 1996; Meier and Reichert, 1990; Yack and Fullard, 1990; Yack and Roots, 1992; Yager and Scaffidi, 1993). Chordotonal sensilla—sometimes singly, but more of-
Chordotonal organs (Fig. 9) are present in the body wall of a locust. The tympanal organ (T1) in the lateral wall of the first abdominal segment (A1) is represented in the other abdominal segments by its serial homologues, the pleural chordotonal organs (pco). In the mesothorax (T2) and metathorax (T3) the serial homologues of the tympanal organ are the wing hinge chordotonal organs (wco); these are associated with a multipolar stretch receptor (sr). The stereotyped organization of the body wall chordotonal organs into three groups persists with some modification into adulthood. It is also seen in Drosophila and is probably an organizing feature for all insects. Modified from Meier T, Reichert H. 1990. Embryonic development and evolutionary origin of the orthopteran auditory organs. J Neurobiol 21:592–616.

Anatomical studies comparing tympanate insects with closely related, but atympanate species have identified tympanal organ precursors in several taxa. Based on location, structure, and innervation pattern, Yack and Fullard (1990) established the homology between the tympanal chordotonal organ in a noctuid moth and a similar organ in an atympanate saturniid moth. They used neurophysiological recording to show that the chordotonal homologue of the saturniid responds to elevation or depression of the hindwing, and apparently functions as a proprioceptor at the wing hinge. Yager and Scaffidi (1993) identified the homologue to the mantis tympanal organ in cockroaches as well as the serial homologue in the atympanate mesothoracic segment of the mantis. The precursor is almost identical in structure and neuron number, but differs from the tympanal organ in the geometry of its attachments. In this case, behavioral experiments (Yager and Tola, 1994) suggest that the precursor is an extremely sensitive vibration detector, and there is further evidence linking its vibration sensitivity to a non-cercal escape system in the cockroach (Pollack et al., 1994). Several comparative studies—both within and among species—show that a group of mechanoreceptors associated with the subgenual organ, also a vibration detector, is clearly the precursor of the enniiferan crista acustica (Kalmring et al., 1994; Lakes-Harlan et al., 1991; Lin et al., 1994). Finally, the precursor of the tachinid fly tympanal organ has been identified in a broad sampling of higher Diptera (Edgecomb et al., 1995; Robert et al., 1996a). The precursor lies in the same location and has a similar structure, but has fewer sensilla (<40 vs. 70). Its function is unknown, although it is most likely a proprioceptor.

The most potent demonstration of tympanal organ precursors comes from developmental studies. Meier and Reichert (1990) used neuron-specific antibodies to trace the embryonic development of the locust tympanal organ on the lateral wall of the first abdominal segment (Fig. 9). The tympanal chordotonal organ is formed at approximately 40% of embryonic development by invagination of the body wall near the posterior border of the segment. From the outset, the organ has its adult number of 70–90 sensory neurons. The pleural chordotonal organs form at the same developmental time, by the same process, and at the homologous locations in the other pregenital abdominal segments. Cell migration and growth of the afferent nerve are largely identical for the two organ types, but the pleural chordotonal organs have only 10–15 sensory neurons. By comparison, they found that an earless grasshopper forms a normal pleural chordotonal organ in the first abdominal segment. Thus, it is clear that the chaeliferan ear derives from the pleural chordotonal organ of the first abdominal segment and that it has serial homologues throughout the thorax and abdomen. Pleural chordotonal organs appear to monitor respiratory movements (Hustert, 1975; Orchard, 1975).

Using the same developmental techniques, Meier and Reichert (1990) also showed that the crista acustica in the prothoracic leg of bushcrickets derives from a linear array of mechanoreceptors associated with the subgenual organ. There are unspecialized serial homologues in the other pairs of legs, and they found that the “crista acustica” in the prothoracic leg of an atympanate species was unspecialized like the organ in the mesothoracic and metathoracic legs. Not all of the sensory neurons are present initially, but the number increases with development. The adult crista acustica has 30–40 sensilla; the serial homologues have 10–15. An especially intriguing finding was that the linear array of sensilla reflecting a tonotopic organization in adults is present even when the receptors first appear in the embryo.

Lewis and Fullard (1996) looked at development of the noctuid moth tympanal organ across metamorphosis. They showed that the tympanal organ does not develop de novo during metamorphosis, but, rather, is the post-metamorphic derivative of a proprioceptor present in the larvae. In contrast to most other cases, the adult tympanal organ has fewer rather than more chordotonal sensilla than its precursor (2 vs. 3; this is
true both compared to the larval precursor and to the homologue in an atympanate moth.

The pneumorid grasshoppers provide an especially interesting and complementary perspective on tympanal organ precursors. Because of their phylogenetic position among the chaeliferida and because of the homology of the pneumorid auditory organs with other chaeliferan ears, van Staaden and Römer (1998) argue that the atympanate, but highly sensitive, serially homologous auditory organs of pneumorids represent a transitional form between the pleural chordotonal organ precursors and fully tympanate auditory systems. In summary, the tympanal organs of all tympanate insects studied have chordotonal precursors and have not arisen de novo. Tympanal organs have many more sensilla than their precursors except in mantids (the same) and noctuid moths (fewer). Some of the precursors are proprioceptors monitoring slow body movements. The normal function of others seems to be indirectly exteroceptive by picking up substrate (and/or air?) vibrations transmitted through cuticle.

**Determinants of Body Location.** Although the diversity of ear locations on the insect body may seem unpatterned and almost random, recent comparative and developmental studies have opened the way for a simpler and more orderly view. Raff (1996) provides a superb examination of these concepts that guide the rediscovered field of "evolutionary developmental biology"; Kutsch and Breidbach (1994) provide a related discussion focusing on invertebrates. For the evolution of insect ears, the key lies in "modularity," not only the modular construction of the insect body, but the underlying modularity of developmental processes. There is also a hierarchy of modules. For example: the insect body is constructed of a series of repeated modules (body segments); within each of the three thoracic segments, a modular unit of gene expression and induction directs the formation of a leg on both sides; within each leg module there are separate anterior, posterior, distal, and proximal modular processes (Tabin, 1991). Thus, the same developmental module in different body segments produces serially homologous components; in this sense, serial homologies are the same structure (Haszprunar, 1992; Wagner, 1989).

The duplication process—multiple segments built by the same sequence of gene expression and induction—is often accompanied by divergence, alteration through natural selection of some modules of particular body segments (Raff, 1996). For instance, the prothoracic legs of mole crickets are specialized for digging and are quite different in structural detail (but not fundamental design) from the serially homologous walking legs. The most conserved elements among body segments are the CNS and the peripheral innervation patterns (Dumont and Robertson, 1986; Meier et al., 1991; Thomas et al., 1984). Thus, even in the highly fused ganglionic mass of the moth thorax, the segmental, modular CNS architecture is preserved (Boyan and Ball, 1993; Boyan et al., 1990). A more extreme example: even though the adult anatomies are radically different, the serial homology of insect mouth parts and legs is obvious in the remarkable similarity of their sensory innervation patterns during development (Meier and Reichert, 1991).

With these concepts in mind, it is interesting to look at ears in three different orders and located at three different sites on the body: locusts (lateral A1), tiger beetles (dorsal A1), and noctuid moths (dorsal/caudal T3). Yager and Spangler (1995) argued that a major difference in body configuration obscures the fact that the ears of locusts and tiger beetles are built from homologous parts, with the pleural chordotonal organ as precursor of the tympanal organ in both cases. Evidence from embryology (Meier and Reichert, 1990) and genetics (Bier et al., 1990) has demonstrated the serial homology of the abdominal pleural chordotonal organs with the thoracic wing hinge chordotonal organs (Fig. 9). Yack and Fullard (1990) have convincingly shown that the wing chordotonal organ in T3 is the precursor of the noctuid tympanal organ. Finally, there is substantial evidence that the moth and locust wing hinge chordotonal organs are homologous (Wilson and Gettrup, 1963; Yack, 1992; Yack and Roots, 1992). Following this chain of logic, three anatomically different ears in unrelated taxa are nonetheless closely allied in an evolutionary/developmental sense. These three independent ear evolutions form an "auditory cluster" that may additionally count among its members the little-studied auditory systems of some aquatic hemipterans (on lateral mesothoracic and metathoracic segments; Prager, 1976) and uraniid moths (on lateral A2; Scoble, 1995) Other auditory clusters may include mantids/tachinid flies (mid-ventral) and cicadas/pyralid moths/geometrid moths (ventrolateral). A more surprising—and speculative—cluster would be based on the serial homology of insect mouth parts with the legs (Meier and Reichert, 1991); it would include sphingid moths and the ensiferans. Finally, the several occurrences of wing ears would form a separate cluster (complicated, however, by the growing evidence that wings are derived from legs and share the same developmental patterns, although some of the genes differ; Williams and Carroll, 1993).

There are two especially notable examples that reinforce the segmental equivalence of ear locations and its potentials. Without doubt, the most striking case is the six serially homologous pairs of auditory organs found in the abdominal segments of pneumorid grasshoppers (discussed above; van Staaden and Römer, 1998). In this instance, the auditory information from all the ears seems to be integrated in service of the same behaviors, mate attraction and courtship. Second, mantids in the hymenopodid subfamilies Hymenopodinae and Acromantinae have two ears (an "auditory bicyclops"; Yager, 1996a). One of these is in the metathoracic midline and has the characteristics described above for other mantids. The second is a serially homologous ear in the mesothorax that has equal or greater sensitivity, is tuned to 2–4 kHz rather than ultrasound, functions independently of the metathoracic ear, and mediates different behaviors. In essence, these insects have evolved two separate auditory systems. In an odd way, pneumorid grasshoppers and hymenopodid mantids are their own auditory clusters.

A corollary to the "auditory cluster" hypothesis is that there are locations on the insect body that are especially likely sites for the appearance of an ear. The limiting determinant is most likely the presence of a neural precursor to the tympanal organ rather than availability of a trachea for the tympanal air sac or suitable cuticle for the tympanum itself. Chordotonal
organs are numerous and found throughout the body, but they are not evenly distributed. Embryological studies clearly show a stereotyped pattern of three chordotonal organ groups (ventral, lateral, and dorsal) in each thoracic and abdominal body segment (Fig. 9). A remarkably similar pattern is found in locusts and in Drosophila (Ghysen et al., 1986; Meier et al., 1991) and, judging by adult anatomy (Finlayson, 1976) and phylogenetic history (Carpenter, 1992), is shared by most, if not all insects. The embryological distribution persists in the adult with two modifications that are also common to most species across a broad taxonomic span (a compilation is provided in Finlayson, 1976). The dorsal group often does not persist in the adult as a chordotonal organ; it may be the precursor of multilobar stretch receptors found in each segment (Heathcote, 1981; Meier et al., 1991). The ventral group typically gives rise to separate ventromedial (proximal) and ventrolateral (distal) COs (Finlayson, 1976; Meier et al., 1991). There can be further subdivision and elaboration, especially of the ventromedial group. Slifer (1936) found four ventromedial COs with a total of 72 scolopidia in a grasshopper rather than the 1—2 COs (6—10 scolopidia) present during development (Meier et al., 1991).

The distribution of chordotonal organs in adult insects predicts three “hot spots”—ventromedial, ventrolateral, and lateral—and those locations correspond to the observed auditory clusters of species with ears located on the body.

Why a particular insect bases its ear location on one chordotonal organ group and not another may have less to do with availability of ear precursors than it does with constraints imposed by the animal’s natural history and anatomical specializations. Considering relatively homogeneous lineages, ears have evolved twice among the parasitoid flies and twice among sphingid moths, in both cases at the same location (other examples are the midline ears of mantids and thealar ears of butterflies). As a counterexample, scarab beetles and tiger beetles have very different ecologies and body shapes, and ears that appeared in different locations.

The lateral and ventrolateral groups in segments T2—A2 are by far the most common precursors possibly because those locations maximize availability of directional cues and at the same time afford protection for the tympanum. An indirect flight mechanism and a shortened abdomen might dictate that the dipteran ear could not use the lateral and ventrolateral body wall CO groups, and therefore a ventromedial location became most practical. All other things being functionally equal, simple fortuity may also be a sufficient explanation.

**Earless-to-Eared Transition.** The central question in the evolution of insect auditory systems—What changes in developmental programs lead to the evolutionary transition from earless to eared?—remains unanswered. The tools for uncovering the answer will come from molecular genetics and evolutionary developmental biology. The genetic and molecular control of chordotonal organ development is now understood in considerable detail for Drosophila (Hartenstein, 1988; Jan and Jan, 1994; Okabe and Okano, 1997). For instance, certain mutations of the engrailed gene lead to absence of the pleural chordotonal organs without affecting any other sensory structures in the region (Hartenstein, 1987). The serial homology of abdominal pleural COs and the thoracic wing hinge COs (Fig. 9) has been confirmed using a mutant of the rhomboid gene that can change the segmental specification of a pleural CO from abdominal (five scolopidia) to thoracic (three scolopidia; Bier et al., 1990). More recently the induction of chordotonal organ precursors by sequential action of the genes atonal, rhomboid, and argos has been described (Okabe and Okano, 1997). It is especially interesting from the evolutionary perspective that the proneural gene atonal directs the formation of chordotonal organs in Drosophila (Jarman et al., 1995) and that the mouse homologue of atonal directs the formation of sensory structures in the inner ear (Bermingham et al., 1999). Although nothing is yet known about the genetic control of tympanal organ development in any insect, the information from Drosophila clearly provides a strong groundwork.

The evolution of insect ears involves more than just the chordotonal organs. At minimum, some process or processes should: (1) trigger the increase in sensory neuron number compared to the chordotonal precursor that is seen in most, but not all, tympanate insects; (2) trigger thickening of a region of cuticle or enlargement of an existing patch of membranous cuticle; (3) trigger thickening of a nearby trachea or migration of an existing tracheal sac to the tympanum; and (4) trigger a reorientation of the three ear components so that they are in the proper spatial relationship to one another.

The ontogenetic transition from earless to eared takes place in stages that may provide clues to the evolutionary processes. In every hemimetabolous insect studied, the neural components of the ear are in place at hatching, although there may be some reorientation postembryonically as nearby structures mature (Klose, 1991; Meier and Reichert, 1990; Rössler, 1992; Yager, 1996b). Nonetheless, the youngest nymphs do not hear. A later, second stage of auditory development builds the tympanum and brings an impedance-matching tracheal sac into apposition with it. For crickets, the appearance of auditory function is abrupt. Last instar nymphs have no appreciable sensitivity; the tympanum appears at the imaginal molt and with it come low thresholds (Ball and Hill, 1978). For tettigonids (Rössler, 1992), locusts (Petersen et al., 1982), and praying mantids (Yager, 1996b), the transition is more gradual. For instance, mantids first have detectable auditory sensitivity about half way through nymphal development, and it increases with each successive instar. The progressively lowering thresholds parallel the enlargement and thinning of the tympanum and the formation of the tympanal tracheal sac. Fully adult ear structure and sensitivity appear at the last molt. Harron and Yager (1996) have shown that the second stage of ear development can be selectively disrupted by appropriately timed doses of juvenile hormone; the outcome is deaf and short-winged, but otherwise normal adults.

**Looking Ahead**

Based on the discussions above, there are some generalizations that can help guide the search for developmental and evolutionary processes in insect audition:
Ears and auditory systems have not arisen de novo, and we can focus on changes in the development of known precursors.

We do not need to look for 19 different evolutionary/developmental mechanisms. The ideas underlying auditory clusters suggest at most 5–6 mechanisms. If, as seems probable, the different body wall chordotonal organ groups have parallel developmental processes, the number may go down to three: body, wing, and leg.

The number could be one. There may be an interesting parallel with the study of eye evolution in metazoans. Anatomically-based phylogenetic studies suggested at least 15 independent evolutions of lensed eyes and 40–65 appearances of photoreceptors in six metazoan phyla (Salvini-Plawen and Mayr, 1977). However, the discovery of a “master control gene” (eyeless and its homologue Pax-6) triggering a highly homologous gene cascade determining eye morphogenesis in both vertebrates and invertebrates argues strongly for a common developmental origin (reviewed in Halder et al., 1995a). The haunting image of fruit flies with fully differentiated compound eyes on their legs or wings or antennae due to local expression of the eyeless gene (Halder et al., 1995b) inevitably raises the prospect that there may be a corresponding control gene that when turned on, orchestrates the construction of an insect ear, regardless of position on the body.

Anatomical changes in the central nervous system to accommodate audition are likely to be small compared to those in the periphery. The idea that the CNS is far less plastic than the periphery has emerged as a general principle in invertebrate neurobiology (Boyan and Ball, 1993; Dumont and Robertson, 1986). We know, for instance, that each insect auditory system uses the same mechanoreceptive region (mVAC) of CNS neuropile regardless of the location of the ear or the nerve root carrying the auditory information (discussed above). Yager and Hoy (1989) argued that the most prominent auditory interneuron in mantids (MR-501-T3) is the homologue of a well-studied auditory interneuron in locusts (531; also called “B1”), as well as of a nearly identical cell (DPG 502) in cockroaches shown by Ritzmann et al. (1991) to receive vibratory input from the subgenuals organs. The implication is that both mVAC and MR-501-T3/531/DPG 502 are components of an ancient chordotonal mechanoreceptive system that has been coopted repeatedly for audition (Boyan, 1993).

This does not, however, mean that the auditory CNS is devoid of interest. On the contrary, one of the most important unanswered questions about the evolution of insect auditory systems concerns CNS changes. With the advent of hearing, portions of the CNS that had processed proprioceptive information begin receiving exteroceptive information that has a different meaning to the animal and must trigger different behavioral responses. How does the CNS reinterpret the afferent information and link it to appropriate behaviors? While there may not be gross anatomical change, clearly some rewiring is necessary. Least likely is the creation of entirely new circuitry and entirely new behaviors. Evolution most often builds from existing structures, and just as there are anatomical tympanal organ precursors, there are certainly precursor neural circuits and behaviors (Dumont and Robertson, 1986). At the other extreme, CNS circuitry and behavior may be entirely modular so that the only change required to accommodate hearing is to disconnect the input from its proprioceptive circuit and reconnect it to, for instance, an escape circuit. Both mantids and tiger beetles provide some support for the modular view: the components of the ultrasound-triggered evasive response in flying mantids are very similar to those of the terrestrial mimic (defensive display) (Yager and May; 1990); the nocturnal response of flying tiger beetles to ultrasound—a fast, strong swing of the elytra into the flapping hindwings—is the major component of the landing response the same beetles use to evade robber flies during the day (Yager and Spangler, 1997). Most of the transformational mechanisms are likely to fall somewhere between the extremes, especially for more complex behaviors like mate attraction and courtship.

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