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Nymphal Development of the Auditory System in the Praying Mantis *Hierodula membranacea* Burmeister (Dictyoptera, Mantidae)

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ABSTRACT

Like other praying mantises, *Hierodula membranacea* has a single midline ear on the ventral surface of the metathorax. The ear comprises a deep groove with two tympana forming the walls. A tympanal organ on each side contains 30–40 scolopophorous sensillae with axons that terminate in the metathoracic ganglion in neuropil that does not match the auditory neuropil of other insects.

Nymphal development of the mantis ear proceeds in three major stages: 1) The tympanal organ is completely formed with a full complement of sensillae before hatching; 2) the infolding and rotations that form the deep groove are completed primarily over the first half of nymphal development; and 3) over the last five instars (of ten), the tympana thicken and broaden to their adult size and shape, and the impedance-matching tracheal sacs also enlarge and move to become tightly apposed to the inner surfaces of the tympana. Auditory sensitivity gradually increases beginning with the fifth instar and closely parallels tympanum and tracheal sac growth. Late instar nymphs have auditory thresholds of 70–80 dB sound pressure level (SPL). Appropriate connections of afferents to a functional interneuronal system are clearly present by the eighth instar and possibly much earlier.

The pattern of auditory system ontogeny in the mantis is similar to that in locusts and in noctuid moths, but it differs from crickets. In evolutionary terms, it is significant that the metathoracic anatomy of newly hatched mantis nymphs matches very closely the anatomy of the homologous regions in adult cockroaches, which are closely related to mantises but are without tympanal hearing, and in mantises that are thought to be primitively deaf.

Indexing terms: ontogeny, evolution, chordotonal organ, hearing, tympanum

Despite the diversity of insect auditory systems and the extensive behavioral and neurophysiological research on some of them, their development has been studied relatively little. Among the tympanate insects with incomplete metamorphosis (hemimetabolous), locust auditory development has received the most attention (Michel and Petersen, 1982; Petersen et al., 1982; Boyan, 1983; Breckow and Sippel, 1985; Meier and Reichert, 1990) followed by that of crickets (Ball and Young, 1974; Ball and Cowan, 1978; Ball and Hill, 1978; Ball et al., 1989) and tettigoniids (Meier and Reichert, 1990). No comparable published reports currently exist for insects with complete metamorphosis (holometabolous). Given their very different developmental patterns, comparisons between these two major groups may prove valuable in understanding the evolution of insect auditory systems.Among the hemimetabolous insects, there appear to be two patterns of auditory system development. In crickets, significant auditory sensitivity first appears after the molt to adult (Ball and Hill, 1978). During postembryonic (nymphal) development, the number of auditory sensillae in the tibiae gradually increases to its adult number of about 70 (Ball and Young, 1974). Recently, Meier and Reichert (1990) have reported that at least some auditory chordotonal sensillae are in place during embryonic development in the tibiae of tettigoniids. Sensitive hearing in crickets is not possible, however, until the thinned tympanum appears in the adult (Ball and Hill, 1978). In fact, the thinning of the tympanum is not complete until several days after the final molt (Ball and Cowan, 1978).

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In contrast, locust nymphs gradually acquire hearing over the last three (of five) instars (Petersen et al., 1982). All of the auditory sensillae are present and are organized in an adult pattern at hatching (Michel and Petersen, 1982; Meier and Reichert, 1990), and a clear auditory neuropil is present by the third instar, when sensitivity to sound first appears (Petersen et al., 1982). The development of the tympanum and associated cuticular structures is progressive, however, and parallels the increase in sensitivity recorded in the auditory nerve of nymphs (Michel and Petersen, 1982; Breckow and Sippel, 1985). Activity in identified auditory interneurons appears with the same time course as activity in the tympanal nerve (Boyan, 1983).

The auditory system of the praying mantis differs considerably from those of other tympanate hemimetabolous insects (Yager and Hoy, 1987). A single ear comprising of two tympana facing each other in a deep groove is located in the ventral midline of the metathorax. The tympanal organ on each side contains 30–40 chordotonal sensillae in *Mantis religiosa*, and the afferent axons terminate ipsilaterally in the metathoracic ganglion in neuropil not known to be auditory in other insects. The majority of mantis species studied have their most sensitive hearing (physiological thresholds of 50–60 dB SPL) between 30 and 60 kHz (Yager and Hoy, 1989; Yager, 1990a). The only currently documented function for hearing in mantises is bat evasion (Yager et al., 1990). Although there is convergence of function, the mantis auditory system has clearly evolved independently from that in other insects. Therefore, the mantis is invaluable in a comparative study of homology (or its absence) in insect auditory systems. This paper documents the physiological and anatomical nymphal development of the mantis auditory system.

MATERIALS AND METHODS

Animals

We chose *Hierodula membranacea* Burmeister (Mantidae, Mantinae) for this study because of its very large size and relative ease of rearing. Our eggs came from a commercial supplier and originated in Sri Lanka. The species was in culture for one full generation prior to the beginning of this study.

Each nymph was reared in a separate container from the time of hatching. We reared a total of 410 nymphs from ten oothecae spanning three generations. The animals were misted with water every day and were fed appropriately sized live food (fruit flies, house flies, or crickets) twice each week. The colony room was maintained at 24–26°C and 30–50% relative humidity with a 14:10 light:dark cycle.

To assess developmental variability both among nymphs and between generations, we monitored the time between molts as well as various size parameters at each instar of each nymph. We also recorded tuning curves from adults of each generation. These data will be reported in detail elsewhere (Yager and Read, in preparation). In brief, we found no greater variability between generations than among individuals of the same generation. The adult tuning curves did not differ significantly (t tests; d.f. = 8–12) across generations.

Anatomy

At each instar, a number of nymphs were removed from the colony to assess the development of their auditory system.

Gross morphology. Nymphs were prepared for scanning electron microscopy (SEM) by fixation in alcoholic Bouin's, dehydration through an ethyl alcohol series, critical-point drying from 100% ethanol, and sputter coating with gold palladium. The specimens were viewed and photographed with an AMR 100A SEM. The metathoraces of unfixed specimens were photographed through a Wild M5 dissecting microscope.

Histology. After 24–48 hours fixation in alcoholic Bouin's, specimens were dehydrated, embedded in paraffin, and sectioned horizontally or transversely at 8 μm. We reduced the difficulties of sectioning insect cuticle by using only freshly molted animals for histology. Slow dehydration in butanol (Lee, 1950) rather than the usual ethanol also proved very effective in keeping tissue soft.

We used a modification of Masson's trichrome to stain the tissue (Pantin, 1946). This technique yields bright, cherry-red scolopale rods and caps and clearly delineates nerve and ligament from surrounding fat and muscle. We sectioned, stained, and examined 13–22 animals for each nymphal instar (omitting the third and fourth instars) and adults for a total of 147 preparations.

Measurements and counts. Body measurements were made on unfixed animals. Tympanal size, however, was determined using animals fixed in Steive fixative (Humason, 1979) or in 70% ethanol. Measurements from histological slides were made with an eyepiece micrometer and have not been corrected for shrinkage due to processing. For soft tissue, this shrinkage may be as much as 20%, but it is likely to be much less for cuticular structures.

We found the number of sensillae in the tympanal organ by counting the scolopale caps and found the number of sensory neurons by counting their nucleoli (there is only one nucleolus in these cells). In both cases, the structures counted are much smaller than the section thickness, thus reducing the incidence of counting the same scolopale or nucleolus twice because it has been split. We have also applied the Abercrombie correction (reviewed in Coggeshall, 1992) to yield a more accurate estimate of scolopale and nucleoli numbers from the serial sections. Most counts were made independently by two individuals to minimize bias effects.

Physiology

We recorded neurophysiological tuning curves on nymphs beginning with the fifth instar, the earliest stage at which any auditory activity could be detected. All recordings were made at least 24 hours after the last molt. We collected data from 12–19 individuals at each instar.

The nymph was affixed with wax to a platform ventral side up, and a wax well was constructed around the pronotum caudal to the prothoracic legs. The cuticle was removed from the ventral pronotum to expose the connectives. The well was kept filled with saline of approximately 340 mOs (Strausfeld et al., 1983). Both connectives were transected near the prothoracic ganglion. In all preparations, the mesothoracic and metathoracic legs were removed at the coxae to eliminate subgenual responses.

We recorded extracellularly using a suction electrode placed over the caudal cut end of the connective ipsilateral to the stimulating speaker. The signal from the electrode was amplified and monitored using conventional electronics. In some cases, experiments were tape recorded (Vetter Model D) for off-line computer analysis.

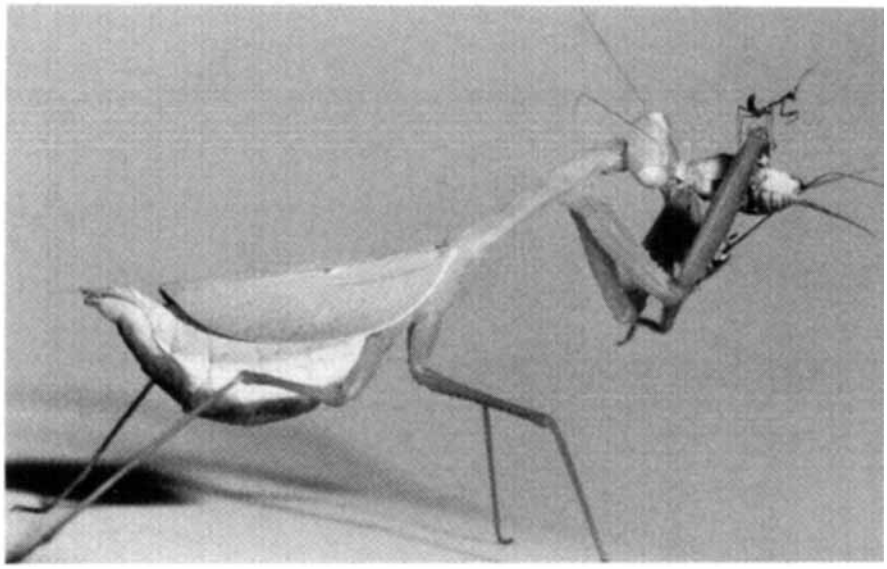

Fig. 1. A second instar *H. membranacea* nymph perched on the raptorial leg of an adult female. The female is eating a cricket. Changes in auditory structure and function reflect both the tremendous growth during nymphal development and the behavioral differences between nymph and adult. The female body length is approximately 90 mm.

All experiments were carried out with the mantis at the center of a 0.6 × 0.6 × 1.85 meter chamber completely lined with acoustic foam to minimize echoes. The speakers (Technics EAS10TH400B leaf tweeters and Realistic 40-1011 woofers) were 0.72 meters from the center of the chamber and 90° to the right and left of the mantis. Temperatures were 21–23°C.

The stimuli were 70 or 100 msec trapezoidal pulses produced by multiplying a sine wave (Tektronix FG-501) with integrated square pulses (Grass S88) using a custom-built 'wave shaper'. Rise and fall times were 3–5 msec. Stimulus amplitude was controlled with a Hewlett-Packard 355D attenuator. The signal was fed to the speakers through a Harmon Kardon PM-655 power amplifier.

We calibrated the stimulus system using a Brüel and Kjaer 4135 6.25 mm microphone (grid off) positioned at the mantis location with the mounting platform in position. Root mean square (RMS) SPLs were provided by a Brüel and Kjaer 2209 SPL meter. Stimulus pulses used for calibration were >80 msec in duration to allow adequate meter rise time. The frequency response of the calibration system is flat ± 1 dB up to 70 kHz. We determined appropriate correction values for frequencies up to 100 kHz by our own calibration of the 2209 meter. Our measurements confirmed attenuator accuracy over the range used and speaker linearity up to 95 dB for frequencies ≤80 kHz (up to 90 dB at >80 kHz). Harmonics with the greatest energy (usually the second) were >35 dB (most were >45 dB) below the fundamental at all frequencies (Nicolet 444A FFT Spectrum Analyzer).

Data analyses

For determining tuning curves, we defined threshold as the minimum SPL eliciting responses in 50% of the trials. Data in decibels were converted to a linear scale (pressure) prior to statistical manipulation, and the results were then converted back to dB for reporting.

Averages are expressed in the text as mean ± standard error unless otherwise noted. The significance level for all statistical tests is 0.05.

RESULTS

General development

Upon hatching, *H. membranacea* nymphs are encased in a loose shell or membrane that they shed within minutes to become first instar nymphs. We have not included the "prenymphs" in this study.

Typically males molt eight times, and females molt nine times after hatching. Thus, males have eight nymphal instars, and females have nine. The times between molts (stadia) range from 9.0 to 25.5 days, with the early stadia shorter than the later ones. The total time from hatch to adult is approximately 105 days for males and 130 days for females. Sex can easily be determined from the external genitalia by the third instar.

The change in body size over the course of nymphal development is substantial (Fig. 1). Length grows by a factor of 8–10 (first instar, 9.5 ± 0.4 mm; adult female, 90.7 ± 3.1 mm). For males, body mass increases from 0.0095 ± 0.001 to 2.08 ± 0.16 g; adult female mass (8.00 ± 1.28 g) is over 800 times that of first instars.

Physiology

Tuning. The tuning of the connective response of adult *H. membranacea* has been described previously (Yager, 1990b). The data presented here from adult siblings of the nymphs in this study do not differ substantially from the earlier work. The adults display a minor sexual dimorphism in hearing (Fig. 2). Females and males are equally sensitive at their best (lowest mean threshold) frequency (t test; d.f. = 17). However, the tuning curve in the male is shifted toward higher frequencies compared to the female. The best frequency for adult males is 50 kHz (vs. 25 kHz for

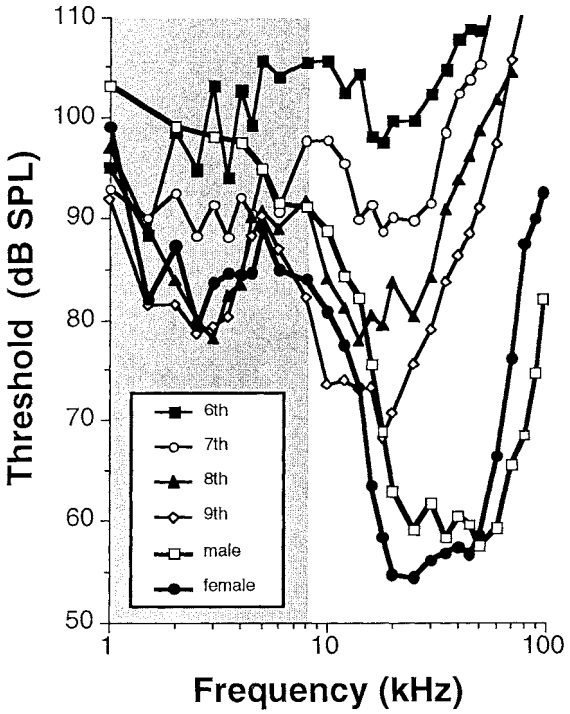

Fig. 2. Physiological tuning curves from connective recordings for different instar nymphs (thin lines) and adults (thick lines). Fewer than half of the fifth instar nymphs tested showed any response to sound. Each curve is the mean of data from ≥12 individuals. Mean standard deviations in the most sensitive frequency regions were 3.3–4.4 dB among nymphs and 4.9–7.5 dB for adults. Sensitivity below 8 kHz (gray region) derives from the mesothoracic "ear."

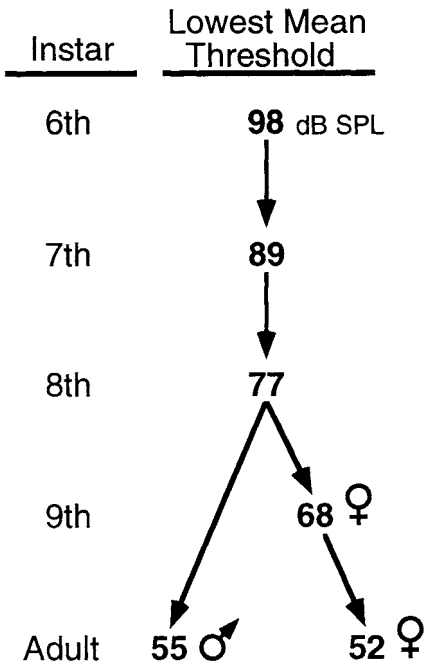

Fig. 3. Schematic diagram showing the pattern of sensitivity change during nymphal development for the two sexes. The largest increase in sensitivity occurs at the last molt, but this change is less for females, because they have one more instar than males. Sensitivity is the same for males and females except at the ninth instar.

females). From 50 to 100 kHz, males are as much as 15–20 dB more sensitive than females, whereas the reverse is true between 10 and 20 kHz. Adult females (but not males) also have moderately low thresholds (75–80 dB SPL) at 2–4 kHz, representing a contribution from a mesothoracic acoustic vibration receptor (Yager, in press).

The earliest we obtained consistent auditory responses in nymphs was the sixth instar (Fig. 2). Of nine fifth instars tested, only four showed responses, and the responses were weak (Fig. 4), with thresholds ≥95 dB SPL. Because our connective recordings assess the responses of auditory interneurons (Fig. 4), these results imply connections between afferents and a functional auditory interneuronal network.

Auditory sensitivity of the metathoracic ear increases gradually from the sixth instar to the adult (Figs. 2, 3). Threshold decreases by 9–12 dB at each molt except at the molt to adult, where the change is much greater and varies by sex. During the sixth, seventh, and eighth stadia, the tuning curves for males and females are identical in both sensitivity and best frequency (t tests; d.f. = 2–9). Males molt from the eighth instar to adults, and sensitivity jumps by an average of 22 dB. In contrast, females molt from the eighth to the ninth instar, and sensitivity increases by only 9 dB, similar to the changes at earlier molts. When the females molt to adults from the ninth instar, however, they also experience a very large increase in sensitivity, although it is smaller than for the males. The net result is that the change in threshold from eighth stadium to adult is the same for males and females; females simply do it in two steps to the males' one.

Tuning also changes with nymphal stage (Fig. 2). Nymphs generally resemble adult females in their tuning. They differ in that they have slightly lower best frequencies (16–20 kHz vs. 20–25 kHz) and narrower best frequency ranges. For males, the transition from eighth instar to adult marks a major tuning change: The entire tuning curve shifts upwards, and the best frequency increases to 50 kHz.

Using the same techniques, we have also recorded the tuning curves of 14 wild-caught *Mantis religiosa* nymphs. Nymphal stage was estimated based on body size and wing bud development. This species showed a pattern similar to *H. membranacea*: increasing sensitivity throughout the last half of nymphal development and an upward shift in tuning.

Connective responses. Based on spike size and firing pattern, the auditory response recorded extracellularly in the ascending connectives of adult *H. membranacea* derives from at least three neurons (Fig. 4). Two (and possibly more) very large spike types appear in a phasic-tonic pattern, and a much lower amplitude unit is more strictly tonic. An initial high-rate burst is followed by sporadic firing throughout a long stimulus pulse. There is a tendency for the spikes in the tonic portion to be clustered into short bursts, just as previously described for another mantis species (Yager and Hoy, 1989). The latency to the first spike at 10 dB above threshold is 18.3 ± 0.4 msec (eight measurements from each of three animals). There is moderate habituation to repeated stimuli, but the response never disappears entirely.

In the few fifth instars that showed any response to sound, there appears to be only a single unit active (Fig. 4).

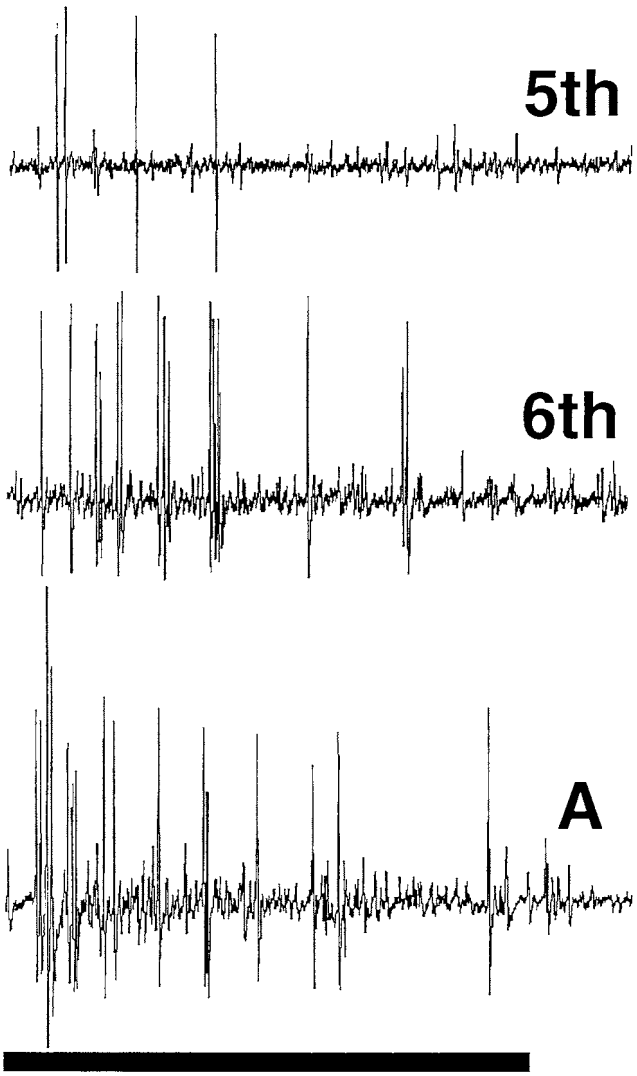

Fig. 4. Extracellular recordings from the connective ipsilateral to the speaker in fifth and sixth instar nymphs and in an adult. The stimulus (black bar) was a 300 msec tone at the best frequency for the individual animal and at 10–15 dB over threshold (for fifth instars, this put the stimulus at > 100 dB SPL). Responses from eighth and ninth instars appear identical to adult responses.

Its firing rate is low, and it is only very weakly tonic. The latency, at 10 dB above threshold, is long and highly variable (26.3 ± 9.2 msec; 13 measurements from one animal).

Sixth instar nymphs display a much more adult-like response pattern (Fig. 4). At least three units are evident, and there is firing throughout most of a 300 msec stimulus. The latency, at 10 dB above threshold, is 20.2 ± 1.9 msec (at least eight trials from each of two animals). These nymphal responses differ from adult responses in two prominent ways: 1) The clustering of spikes into bursts is more marked throughout much of a long (300 msec) stimulus than in the adult, but the initial burst is not as strong; and 2) repeated stimuli cause greater habituation in the nymph than in the adult. In fact, the response may disappear entirely after several long stimulus pulses. Seventh instars closely resemble sixth instars, and the eighth and ninth instars are almost indistinguishable from adults in their connective responses.

Anatomy

Nonneural structures. The metathoracic ear of *H. membranacea* is almost identical to that described previously for *Mantis religiosa* (Yager and Hoy, 1987). It comprises a deep, longitudinal, midline groove at the posterior border of the metathorax (Figs. 5, 6). Two very prominent cuticular knobs demarcate the anterior end of the groove. The furcasternum forms the floor of the groove, and the furcal pits are deep in the groove near its posterior end. The walls of the groove face each other, and each contains a tear-drop-shaped tympanum of very thin but stiff cuticle. The tympanum is bounded ventrally by a narrow ridge of cuticle. A large tracheal sac is tightly apposed to the internal face of the tympanum. The overall structure of the adult ear in *H. membranacea* is not overtly sexually dimorphic, but the female ear is slightly larger (Yager, 1990b).

First instar nymphs do not have any groove at all in the ventral metathorax (Figs. 5, 6). The furcasternum runs dorsoventrally at the junction of thorax and abdomen, and the furcal pits are exposed at its base. Lateral to the furcasternum, on each side, there is a posteriorly facing, disc-shaped area of membranous cuticle with a dorsoventrally oriented slit. These two areas will become the walls of the groove, and the slits ("pretympana") will broaden later into the tympana.

Taking the first instar anatomy as the starting point, formation of the adult groove requires rotation around two axes and considerable enlargement of the pretympana (Figs. 5, 6). Rotation around a dorsoventral axis through the base of the furcasternum causes the pretympana to face medially rather than posteriorly. This is accompanied by rotation around a transverse axis (also through the base of the furcasternum) that reorients the pretympana so that the slit is longitudinal rather than dorsoventral. These changes in topology form the groove. The walls of the groove elongate, and the slit broadens to form the tympana. The furcasternum changes in concert: It elongates, and its orientation becomes predominantly longitudinal instead of dorsoventral (Fig. 6).

The second instar metathoracic groove differs from the first instar primarily in that it is larger. The rotations described above take place during the third and fourth stadia, and the basic groove is in place by the fifth stadium (Fig. 5). Changes in the ear during the remainder of nymphal development are primarily in size rather than in shape. In particular, two acoustically important structures, the tympanum and the auditory tracheal sac, enlarge during the last half of nymphal development.

The tympanum undergoes major changes in size, shape, and thickness during nymphal development (Figs. 6, 7). The slit-like pretympanum is a very narrow groove that is 126 ± 6 μm long and < 20 μm wide. In contrast, the adult tympanum has a tear-drop shape, a maximum length of 1,289 ± 15 μm, and a width of 526 ± 10 μm. Figure 7 shows that the lengthening of the tympanum matches the pattern of growth of the body as a whole during the immature stages but that the increase in length is disproportionately large at the molt to adulthood. Tympanal width also grows allometrically until the fifth instar. Thereafter, however, the width increases much faster than body size in general. It is this disproportional increase in width that changes the shape of the tympanum.

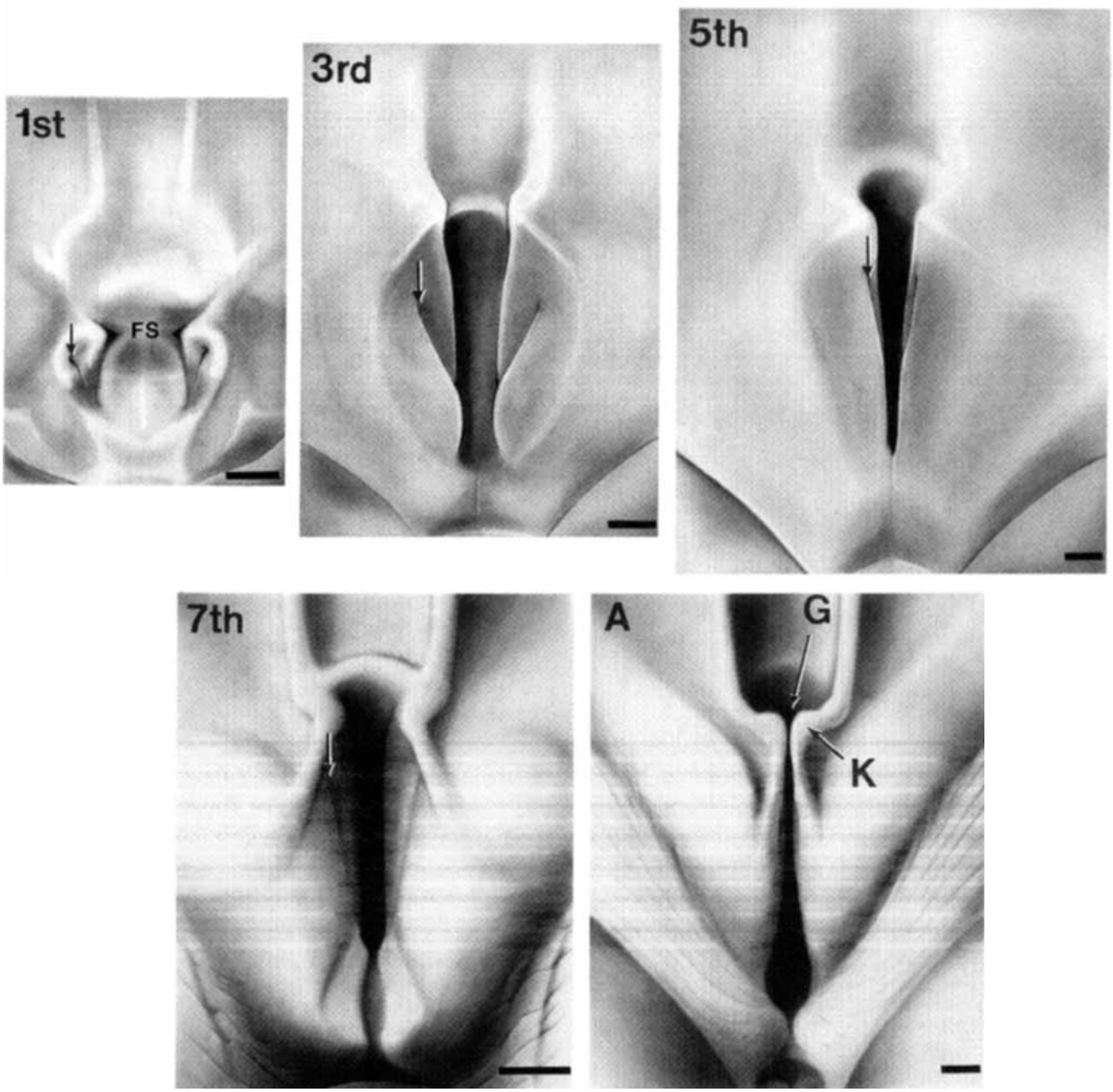

Fig. 5. Changes in the external anatomy of the ventral caudal metathorax (ear region) during nymphal development showing formation of the auditory groove. The arrows in the nymph drawings indicate the rostral end of the pretympanum/tympanum at the attachment point of the chordotonal organ: This is not visible in the adult, because it is deep in the groove. FS, furcasternum; G, deep groove of ear; K, cuticular knob. Anterior is toward the top. Scale bars = 50 μm in first and third instars, 100 μm in fifth instar, 250 μm in seventh instar and adult.

In the adult, the tympanum actually comprises three layers: the cuticle, an epithelial layer one cell thick, and the wall of the tympanal tracheal sac. Because the tracheal sac is not apposed to the tympanum in earlier instars, there are then only two layers. We measured tympanum thickness at the location shown in Figure 8 for second and for fifth through ninth instar nymphs as well as for adults (n = 7–11 at each stage). The tympanal cuticle approximately doubles in thickness during nymphal development (second instars, 2.4 ± 0.2 μm; adults, 4.9 ± 0.2 μm). All of this change occurs progressively after the fifth instar. Like the adults, nymphs have a tympanal epithelium one cell thick, but it is somewhat thinner in nymphs: 4.1 ± 0.3 μm vs. 6.4 ± 0.4 μm. Considering all of the layers, the adult tympanum is 17.9 ± 0.4 μm thick compared to 6.8 ± 0.3 μm for fifth instars.

Auditory tracheal sacs are not present at or before the fifth instar (Fig. 8). In fifth instars, a moderately large

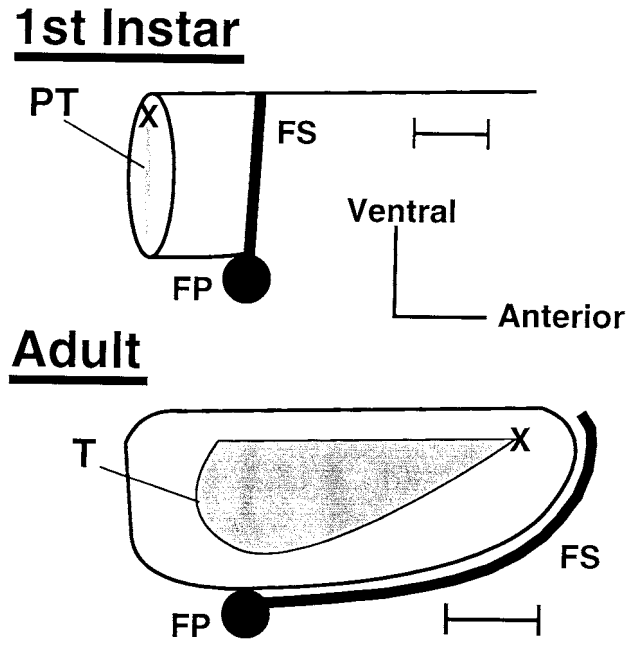

Fig. 6. Schematic midsagittal view of the left caudal metathorax showing the transition from slit-like pretympanum (PT) in the newly emerged nymph to tympanum (T) in the adult. The wall of the ear's deep groove faces medially in the adult, whereas its precursor in the nymph faces posteriorly. The X in each drawing marks the attachment site of the tympanal chordotonal organ. FS, furcasternum; FP, furcal pit. Scale bars = 50 μm in nymph, 400 μm in adult.

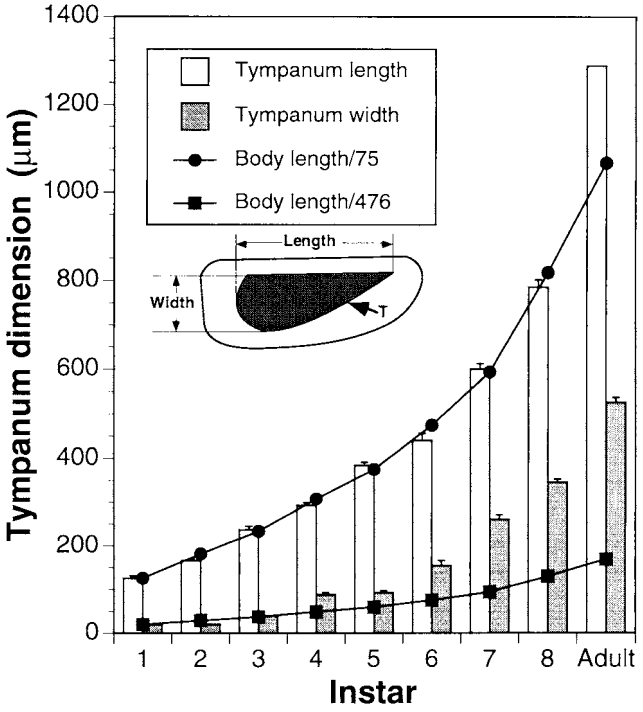

Fig. 7. Changes in maximum dimensions of the tympanum during nymphal development. The **inset** of a groove wall oriented exactly as in Figure 6 shows the measurement axes. Data are for both sexes combined for nymphs and for adult males. Sample sizes were five to ten specimens per instar. Standard errors are shown by the vertical bars. The lines show the body length changes of males during nymphal development scaled so that the value for the first instar equals the maximum tympanal length or width. This demonstrates that tympanal length increases in constant proportion to the body length except for adults. However, tympanal width increases much faster than body length beginning with the sixth instar, which is also when we find the first consistent auditory sensitivity.

tracheal tube passes through the area of the ear, but it is not associated with the tympanum. During the next three stadia, this tracheal tube enlarges and comes to lie closer and closer to the tympanum. By the eighth instar, a true tracheal sac is present and is tightly apposed to the tympanum over at least some of its area. In adults, the area of apposition includes almost all of the inner surface of the tympanum.

Neural structures. The tympanal organ of adult *H. membranacea* (Figs. 9, 10) comprises 34.9 ± 0.9 (n = 11) chordotonal sensillae based on scolopale counts (38.8 ± 2.1 based on somata counts; n = 7). Males and females do not differ (t test; d.f. = 9). The bipolar neuron somata are clustered together in the tympanal organ proper. The scolopales are all oriented with the caps away from the cell bodies and are associated with three ligaments: one attaches to the tympanum, and the other two attach to the ventral body wall laterally. The scolopales in the tympanal ligament are oriented 180° to those in the lateral ligaments. Axons of the bipolar auditory neurons travel to the metathoracic ganglion in the tympanal nerve.

The overall organization of the tympanal chordotonal organ and its ligaments is very similar in early instars and in adults (Fig. 9). The geometry and proportions differ, however, reflecting the rotations and enlargement that occur during development. In the first and second instars, the tympanal organ and its ligaments run longitudinally, and the entire structure is very short. The "X" in Figure 6 indicates the attachment site of the tympanal ligament on the pretympanum and the adult tympanum. While the groove forms, the tympanal attachment site is "dragged" medially, and the enlargement of the groove requires that the tympanal organ elongate. Thus, in the late instars, the tympanal organ is quite stretched out compared to the first instars and is oriented more transversely than longitudinally.

The numbers of scolopales and neuron somata do not change appreciably during nymphal development (Fig. 10). We find no differences in corrected scolopale counts from the second stadium to the adult either for the tympanal ligament alone or for the total counts [analysis of variance (ANOVA); d.f. = 72]. Our data show that first instar nymphs have only about 70% the number of scolopales compared to later instars, a statistically significant difference (t test; d.f. = 83). These lower counts, however, are most likely an artifact resulting from tympanal organ size and geometry. The tympanal organ in first instars is compressed, so that there is considerable overlap of cells and scolopale caps. This makes it extremely difficult to get accurate counts and almost certainly results in an underestimate. The somata counts parallel the scolopale counts (Fig. 10) and indicate that a single neuron is associated with each scolopale throughout nymphal development.

We also measured scolopale cap size at the second and fifth instars (five animals each) and in adults (seven animals). Tympanal and lateral ligament scolopale caps do not differ from each other at any stage (t-tests; d.f. = 29).

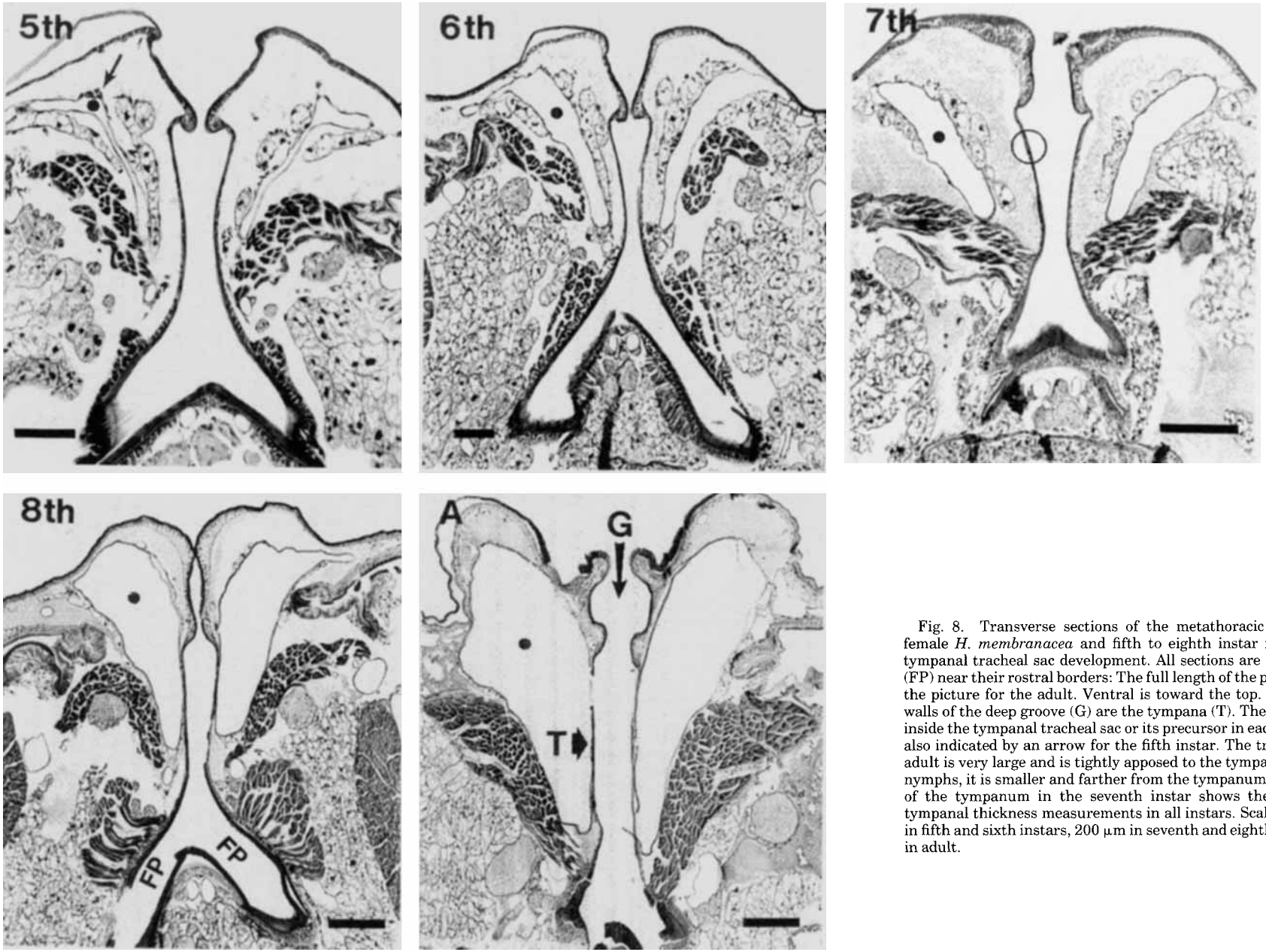

Fig. 8. Transverse sections of the metathoracic ear of an adult female *H. membranacea* and fifth to eighth instar nymphs showing tympanal tracheal sac development. All sections are at the furcal pits (FP) near their rostral borders: The full length of the pits extends out of the picture for the adult. Ventral is toward the top. In the adult, the walls of the deep groove (G) are the tympana (T). The solid black dot is inside the tympanal tracheal sac or its precursor in each section. This is also indicated by an arrow for the fifth instar. The tracheal sac in the adult is very large and is tightly apposed to the tympanum. In younger nymphs, it is smaller and farther from the tympanum. The circled area of the tympanum in the seventh instar shows the location of the tympanal thickness measurements in all instars. Scale bars = 100 μm in fifth and sixth instars, 200 μm in seventh and eighth instars, 250 μm in adult.

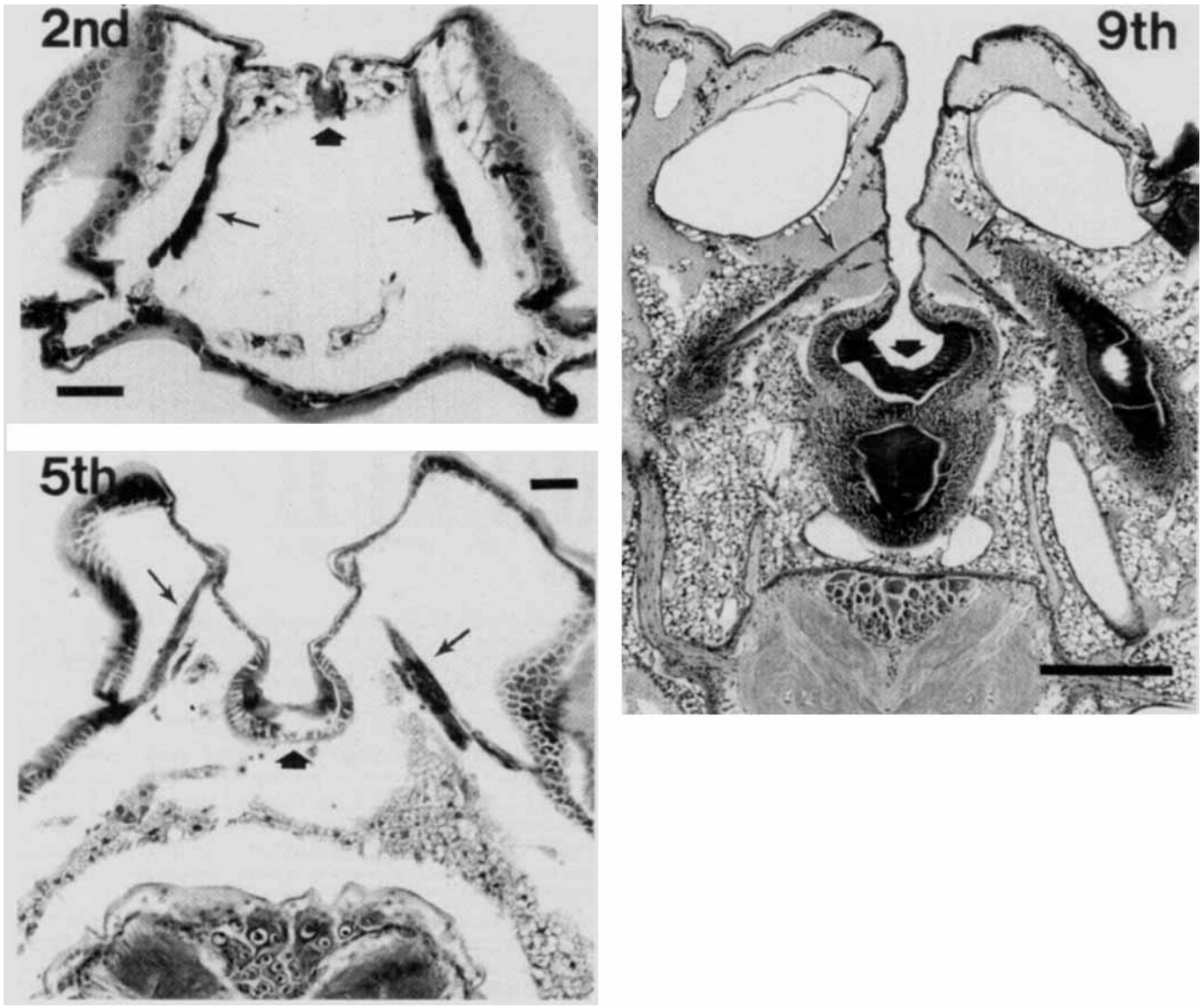

Fig. 9. Horizontal sections of nymphal 'ears' showing the relationship of the tympanal organ to the tympanum as the deep groove forms. The thin arrows point to the tympanal organ/ligament. The thick arrows indicate the furcasternum at the base of the developing groove. Caudal is toward the top. The metathoracic ganglion is visible at the bottom of the fifth and ninth instar sections. While the walls of the groove progressively fold inward, the orientation of the tympanal organ goes from longitudinal to almost transverse. The plane of section is very ventral and excludes most of the tympanal tracheal sacs in the ninth instar. Scale bars = 50 μm in second and fifth instars, 400 μm in ninth instar.

Scolopale width did not vary with developmental stage (ANOVA; d.f. = 30). Adult scolopale length was slightly, but significantly, greater than fifth instar scolopales (3.10 μm vs. 3.46 μm; t-test; d.f. = 18). Because the resolution of our measurement technique is approximately ±0.2 μm, this difference is unlikely to be biologically significant. Using all of the measurements to compute means, the scolopale caps are 1.9 ± 0.1 μm wide and 3.3 ± 0.1 μm long (n = 31).

Yager and Hoy (1987) reported an unusual conical sensillum of unknown function located at the anterior end of the tympanum. It is innervated by two scolopophorous sensillae. We found this structure in three out of eight second instars and in all older nymphs but did not see it in first instars. It was always associated with two scolopales with large caps (3.9 ± 0.1 μm wide by 3.5 ± 0.1 μm long; n = 13).

DISCUSSION

Nymphs of the mantis *H. membranacea* gradually acquire hearing so that even before their final molt, they have moderate sensitivity (70–80 dB SPL) at their best frequencies of 15–25 kHz compared to adults. The molt to adulthood confers a large increase in sensitivity and, at least for males, tuning to substantially higher frequencies than nymphs. In terms of the patterns of auditory development among the hemimetabolous insects, this matches the

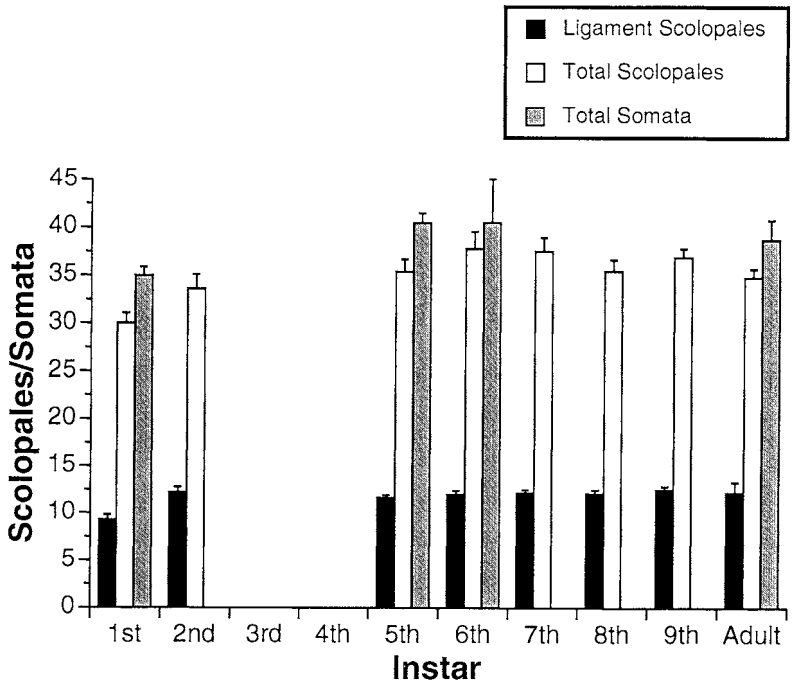

Fig. 10. Mean counts of scolopales and of neuron somata in the tympanal chordotonal organ during nymphal development. Ligament scolopales are only those in the ligament attaching to the tympanum–generally the easiest and most consistent counts. No data were collected for third or fourth instars. There are no statistically significant changes between instars, except that all counts for the first instar are lower than later stages. This difference likely reflects differences in the geometry of the tympanal organ rather than lower actual numbers of sensillae.

pattern seen in acridids (Michel and Petersen, 1982; Petersen et al., 1982; Boyan, 1983) but is quite different from that reported for crickets (Ball and Young, 1974; Ball and Hill, 1978; Ball et al., 1989).

Deaf-to-hearing transition

The progression of auditory development is not uniform over nymphal development: It does not begin until the fifth instar, and it then proceeds gradually. Matching developmental timetables for the various components of the auditory system can suggest the most critical functional changes in the development of hearing.

The neural components of the peripheral auditory system appear to be in place at hatching, and the organization of the tympanal organ matches that of the adult except for small variations in geometry. Not only are the numbers of sensillae the same, but the neurons and scolopales appear under the light microscope to be fully developed. Nevertheless, this does tell us when they actually become functional: a question that can only be resolved by tympanal nerve recordings. Such recordings would be especially interesting, because they might show sensillae functioning perfectly well but for purposes other than audition.

Our connective recordings do allow us to say that, by the eighth instar, the tympanal organ is functioning and that the afferents are making appropriate connections to a functional system of auditory interneurons. At least some of the afferent connections are made earlier, but our present data do not allow us to asses the extent of those connections or the maturation of the auditory interneurons. In the case of the locust, the auditory neuropil is present very early in nymphal development (Petersen et al., 1982), and Boyan (1983) has found that important auditory interneurons have their adult shapes and neural properties throughout postembryonic development. In both cases, changes after hatching appear to be predominantly allometric growth. Juvenile hormone levels are known to play a significant role in the development and the functional maturation of interneurons in crickets (Stout et al., 1991; Carye et al., 1994).

The various nonneural components of the auditory system each follow its own developmental timetable. The infolding and rotations that form the deep groove, for instance, begin with the second instar and are largely completed by the sixth instar, before the first consistent functional evidence of hearing appears. Thus, it is unlikely that the groove itself is the limiting component for auditory sensitivity. Physiological evidence does suggest that placing the tympana in a deep, narrow groove confers an increased sensitivity of almost 5 dB (Yager and Hoy, 1987), which, in part, could account for the large drop in threshold at the final molt, when the groove is fully formed.

Although tympanum length increases allometrically throughout nymphal development, the adult shape does not begin to appear until the sixth instar and is manifested by a disproportional increase in width. Following a parallel time course, the tympana also increase in thickness. Although the role of increased tympanal surface area in improving auditory reception is evident, the thickening is surprising. The pretympanal cuticle is very flexible and membranous, whereas the adult tympana are quite stiff. This change would most likely affect the tuning of the vibrating system, making it much higher in optimal frequency (Michelsen and Nocke, 1974). Especially given the unusual attachment site of the tympanal organ at the extreme anterior corner of the tympanum, we cannot rule out stiffness as a prerequi-

site for functioning of the transduction mechanism (and not just for tuning).

Also closely paralleling the time course of sensitivity increase is the development of the tympanal tracheal sacs. An air space behind the tympanum is necessary for effective acoustic impedance matching, which, in turn, is a major determinant of overall sensitivity (Michelsen and Nocke, 1974). The sacs not only increase dramatically in size, but they also become more and more tightly apposed to the tympanum.

In summary, development of the peripheral auditory system proceeds in three stages: 1) The tympanal organ develops prior to hatching; 2) the infolding and rotations shaping the groove take place largely during the first half of nymphal development; and 3) the formation of the tympana and tympanal tracheal sacs occurs during the last half of nymphal development. Thus, the timetable for hearing acquisition is most likely governed by the completion of nonneural components and not by neural constraints. This is also true for acridids (Michel and Petersen, 1982; Breckow and Sippel, 1985) and even for crickets, because the tympanal organ is in place by the last instar, but effective hearing only appears with the appearance and maturation of the tympana in the adult (Ball and Cowan, 1978; Ball and Hill, 1978).

Behavioral considerations

Immature hemimetabolus insects share a general body plan and many aspects of their general ecology with adults (Davies, 1988). Nevertheless, juvenile mantises differ from their adult counterparts in ways that should strongly affect their auditory behavior and ecology. Most obviously, nymphs are nonreproductive, eliminating any possible requirement for audition in courtship and mating. Young mantises are likely to have different predators than adults, because they are smaller, they may frequent different microhabitats, and they do not fly. The last is particularly important, because the only documented function for audition in adult mantises is evasion of flying, echolocating bats (Yager et al., 1990). In fact, adults without wings generally have reduced or absent hearing (Yager, 1990a,b). In light of the possible sensitivity to vibration (see below), a developing nymphal ear might well function in a substrate vibration-based (as opposed to an airborne vibration-based) escape system. However, we cannot disregard the possibility that the decreasing thresholds to sound in late instar nymphs simply reflect a transitional phase in the development of the auditory system and do not have specific behavioral consequences.

Immature holometabolous insects do not share either a general body plan or an ecology with adults (Davies, 1988). Despite the contrast with hemimetabolous insects, it may be that, in overall pattern, auditory development is similar in moths and mantises (and possibly in acridids). Lewis and Fullard (personal communication) have shown that lymantriid moth larvae do not have an ear but do have an ontogenetic homolog of the tympanal organ in place that may function as a proprioceptor at the intersegmental boundary rather than as an auditory organ as it does in the adult. Thus, the neural and peripheral machinery for a specific, nonauditory function are present at hatching and are used during early development; there is a transitional period during which the periphery (and the central nervous system?) is reshaped; and the same neural structure then emerges as a tympanal organ in the adult. Mantises exhibit a very similar pattern, except that the transitional period is the last half of nymphal development rather than pupation. An important ontogenetic and evolutionary question, then, is what role the mantis "tympanal" chordotonal organ plays during the first four or five stadia.

Evolutionary considerations

To the extent that we can draw inferences about pathways of evolutionary change from ontogenetic patterns (Gould, 1977; Wiley, 1981), mantis auditory development may provide significant clues to the evolution of the unique mantis ear. A recurrent theme in studies of insect sensory system evolution is that the non-neural periphery is much less conservative than the neural periphery (Dumont and Robertson, 1986; Meier et al., 1991). In the case of the mantis, the transition from deaf to hearing, both ontogenetic and evolutionary, may be attributable solely to biomechanical changes: the broadening and thickening of the tympana and the apposition of large tracheal sacs to their inner surfaces. We cannot as yet address the very interesting question of what happens in the central nervous system with the acquisition of hearing, but we predict that the central nervous system will also prove very conservative.

Three anatomical comparisons of first instar nymphs also suggest evolutionary relationships. In gross anatomy, the ventral metathorax between the third legs is very similar in first instar mantis nymphs, in adult cockroaches (Yager and Scaffidi, 1993), and in adult mantises of species thought to be primitively deaf (Yager, 1990a). Serial homology is also evident in the resemblance between the mesothorax and metathorax of newly emerged mantis nymphs and of adult cockroaches (Yager and Scaffidi, 1993). Whereas the mantis metathoracic anatomy changes to become the ear, the mesothoracic anatomy remains constant throughout nymphal development. Given their very close phylogenetic relationship to mantises (Kamp, 1973; Kristensen, 1981), cockroaches are a natural outgroup for evolutionary comparisons. The similarities in metathoracic structure suggest that, in first instar nymphs and in cockroaches, we are seeing the primitive form of the anatomy.

Based on anatomical evidence, Yager and Scaffidi (1993) have suggested that Nerve 7 arising from the cockroach metathoracic ganglion is the homolog of the mantis tympanal nerve. The marked histological similarities we see between first instar nymphs and adult cockroaches further strengthens this argument.

Understanding the primitive function of the tympanal nerve would be a major step toward understanding the evolution of the mantis auditory system. Based on the arguments above, we expect that the primitive function will be reflected in how early instar mantises use their "tympanal" nerve and how adult cockroaches use Nerve 7. Pollack et al. (1995) have provided neural evidence suggesting that Nerve 7 may provide a route for vibrational information to interface with the cockroach escape system. Yager and Tola (1994) have tested behaviorally the function of Nerve 7 using ablation experiments and also found that the chordotonal component of the nerve (but not the component innervating hairs) senses vibration. The idea of vibration detection as the precursor function to hearing reinforces the prediction that the ontogenetic (and evolutionary) central nervous system changes we will find across the deaf-to-hearing transition will be relatively small, because the ear, with its biomechanical adaptations, simply transduces a specialized form of vibration.

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LITERATURE CITED

Ball, E.E., and A.N. Cowan (1978) Ultrastructural study of the development of the auditory tympana in the cricket *Teleogryllus commodus* (Walker). J. Embryol. Exp. Morphol. *46:*75–87.

Ball, E.E., and K.G. Hill (1978) Functional development of the auditory system of the cricket, *Teleogryllus commodus*. J. Comp. Physiol. *127:*131–138.

Ball, E.E., and D. Young (1974) Structure and development of the auditory system in the prothoracic leg of the cricket *Teleogryllus commodus* (Walker). II. Postembryonic development. Z. Zellforsch. *147:*313–324.

Ball, E.E., B.P. Oldfield, and K.M. Rudolph (1989) Auditory organ structure, development, and function. In F. Huber, T.E. Moore, and W. Loher (eds.): Cricket Behavior and Neurobiology. Ithaca, NY: Cornell University Press, pp. 391–422.

Boyan, G. (1983) Postembryonic development in the auditory system of the locust. J. Comp. Physiol. *151:*499–513.

Breckow, J., and M. Sippel (1985) Mechanics of the transduction of sound in the tympanal organ of adults and larvae of locusts. J. Comp. Physiol. A *157:*619–629.

Carye, M., C. Strambi, and A. Strambi (1994) Neurogenesis in an adult insect brain and its hormonal control. Nature *368:*57–59.

Coggeshall, R.E. (1992) A consideration of neural counting methods. Trends Neurosci. *15:*9–13.

Davies, R.G. (1988) Outlines of Entomology. London: Chapman and Hall.

Dumont, J.P.C., and R.M. Robertson (1986) Neuronal circuits: An evolutionary perspective. Science *233:*849–853.

Gould, S.J. (1977) Ontogeny and Phylogeny. Cambridge, MA: Harvard University Press.

Humason, G.L. (1979) Animal Tissue Techniques, Fourth Ed. San Francisco: W.H. Freeman and Company.

Kamp, J.W. (1973) Numerical classification of the orthopteroids, with special reference to the Grylloblattodea. Can. Entomol. *105:*1235–1249.

Kristensen, N.P. (1981) Phylogeny of insect orders. Annu. Rev. Entomol. *26:*135–157.

Lee, B. (1950) The Microtomist's Vade-Mecum, Eleventh Ed. Philadelphia: The Blakiston Company.

Meier, T., and H. Reichert (1990) Embryonic development and evolutionary origin of the orthopteran auditory organs. J. Neurobiol. *21:*592–610.

Meier, T., C. Fabienne, and H. Reichert (1991) Homologous patterns in the embryonic development of the peripheral nervous system in the grasshopper *Schistocerca gregaria* and the fly *Drosophila melanogaster*. Development *112:*241–253.

Michel, K., and M. Petersen (1982) Development of the tympanal organ in larvae of the migratory locust (*Locusta migratoria*). Cell Tissue Res. *222:*667–676.

Michelsen, A., and H. Nocke (1974) Biophysical aspects of sound communication in insects. Adv. Insect Physiol. *9:*247–296.

Pantin, C.F.A. (1946) Notes on Microscopical Techniques for Zoologists. Cambridge: Cambridge University Press.

Petersen, M., K. Kalmring, and A. Cokl (1982) The auditory system in larvae of the migratory locust. Physiol. Entomol. *7:*43–54.

Pollack, A.J., R.E. Ritzmann, and J.T. Watson (1995) Dual pathways for tactile sensory information to thoracic interneurons in the cockroach. J. Neurobiol. *26:*33–46.

Stout, J., G. Atkins, and D. Zacharias (1991) Regulation of cricket phonotaxis through hormonal control of the threshold of an identified auditory interneuron. J. Comp. Physiol. A *169:*765–772.

Strausfeld, N.J., N.J. Seyan, D.W. Wohlers, and J.P. Bacon (1983) Lucifer yellow histology. In N.J. Strausfeld (ed.): Functional Neuroanatomy. Berlin: Springer-Verlag, pp. 132–155.

Wiley, E.O. (1981) Phylogenetics. New York: John Wiley and Sons.

Yager, D.D. (1990a) A diversity of mantis ears: Evolutionary implications. Proceedings of the Fifth International Meetings of the Orthopterist's Society (Segovia, Spain; July, 1989). Madrid: Ministerio de Agricultura Pesca y Alimentation. Boletin de Sanidad Vegetal, No. 20.

Yager, D.D. (1990b) Sexual dimorphism of auditory function and structure in praying mantises (Mantodea; Dictyoptera). J. Zool. (London) *221:*517–537.

Yager, D.D. (1995) Serially homologous ears perform frequency range fractionation in the praying mantis, *Creobroter* (Mantodea, Hymenopodidae). J. Comp. Physiol. Abstr. (in press).

Yager, D.D., and R.R. Hoy (1987) The midline metathoracic ear of the praying mantis, *Mantis religiosa*. Cell Tissue Res. *250:*531–541.

Yager, D.D., and R.R. Hoy (1989) Audition in the praying mantis, *Mantis religiosa* L.: Identification of an interneuron mediating ultrasonic hearing. J. Comp. Physiol. A *165:*471–493.

Yager, D.D., and D.J. Scaffidi (1993) Cockroach homolog of the mantis tympanal nerve. Soc. Neurosci. Abstr. *19:*340.

Yager, D.D., and K.C. Tola (1994) Transection of the mantis tympanal nerve homolog in the cockroach alters resting posture, escape turning, and responses to substrate vibration. Am. Zool. *34:*469.

Yager, D.D., M.L. May, and M.B. Fenton (1990) Ultrasound-triggered, flight-gated evasive maneuvers in the praying mantis, *Parasphendale agrionina* (Gerst.). I: Free-flight. J. Exp. Biol. *152:*17–39.