THE AUDITORY SYSTEM OF AN ATYMPANATE BUSHCRICKET *PHASMODES RANATRIFORMES* (WESTWOOD) (TETTIGONIIDAE: ORTHOPTERA)

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Summary

The auditory system of the atympanate tettigoniid *Phasmodes ranatriformes* has been investigated using anatomical and physiological methods. The prothoracic tracheae show no specialization in terms of an acoustic spiracle, acoustic bullae or an enlargement of the trachea in the proximal tibia. Instead of a true tympanum, a depression with small cuticular protuberances is found on the proximal tibia of the foreleg. The complex tibial organ is formed from the three sensory organs usually found in tettigoniids. The crista acustica consists of about 16–18 receptor cells. Extracellular recordings from the leg nerve reveal sensitive responses to vibrational stimuli (at a threshold comparable to that found in other tettigoniids) only, and not to airborne sounds. The two possibilities either that this animal shows a secondary reduction of the auditory system or that it represents a primitive form are discussed. Developmental data from other tettigoniids seem to confirm the latter hypothesis.

Introduction

The auditory system of the ensiferan Orthoptera is highly developed and remarkably consistent in its form between superfamilies (Otte, 1977; Bailey, 1990). While crickets (Gryllidae) show a variation in terms of the presence or absence of hearing organs, usually associated with the occurrence of sound-producing organs (Otte and Alexander, 1983), there are few reported cases in bushcrickets (Tettigoniidae) of one or both sexes having lost hearing function altogether. One example is in the highly modified hearing system of a stick-like

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insect in the sub-family Zaprochillinae described by Bailey and Simmons (1991). They show that, in the male, the usual opening of the trachea that is linked to the hearing organ in the foreleg is undeveloped, whereas in the female it is fully functional. The tympana of the animals show no sexual dimorphism. The male of this still undescribed genus may hear airborne sound but with a much lower sensitivity than the female (H. Römer and W. J. Bailey, unpublished results).

The ear of most tettigoniids is not only sensitive to airborne sound but also contains receptors sensitive to substratum vibration. The axons of these receptor groups project into the auditory neuropile of the prothoracic ganglion. Vibrational information from these receptors may be used for social communication or as an early warning detector of approaching predators. Substratum vibration, which is transmitted during stridulation, may augment the information provided by airborne sound (Kalmring and Kühne, 1980; Weidemann and Keuper, 1987). In some cases, sound communication between and within the sexes is reduced entirely to substratum vibration; for example, some tettigoniids resort to drumming on the substratum (e.g. *Meconema thalassinum*; Sismondo, 1980), whereas many pseudophyllinae species communicate through tremulations of the substratum (Belwood and Morris, 1987; Morris *et al.* 1989). However, in these non-acoustic species, auditory structures appear to be developed as in tympanate tettigoniids, although the number of receptor cells of the crista acustica may be reduced (Schumacher, 1973, 1979).

This paper describes an unusual loss of hearing in a species of tettigoniid belonging to a monotypic genus endemic to Western Australia, *Phasmodes ranatriformes* (Westwood). We examine the anatomy and physiology of the receptor system, which is associated mainly with the detection of substratum vibration. The means of social communication in this species remains undescribed, but it is possible that substratum vibration may have replaced the role of airborne sound. We discuss the alternative possibilities either that the auditory organ is secondarily reduced in this species or that it represents an evolutionary primitive situation.

Materials and methods

Experiments were carried out on the tettigoniid *Phasmodes ranatriformes*, a genus typified by its extraordinary similarity to stick insects (Fig. 1A). The genus seems to be at least superficially close to another stick-like tettigoniid belonging to the subfamily Zaprochillinae (Rentz and Clyne, 1983; Gwynne and Bailey, 1988). Both sexes of *P. ranatriformes* are apterous and appear to be completely mute. The sexes are highly dimorphic and, again like the phasmids, the female (body length 6.2 cm) is considerably larger than the male (body length 5.3 cm). However, we could find no sexual dimorphism in the anatomy and physiology of the auditory system.

Insects were collected from an area of *Acacia/Eucalyptus/Banksia* woodland called King's Park, close to the University of Western Australia, during September

and October. Material was air-freighted to Philipps University, Marburg, and kept in holding cages at approximately 18°C.

Anatomy of the hearing system

Although our results show a substantial reduction in auditory function compared to that of other tettigoniids, we use the common physiological and anatomical terms for the tympanic organs, underlining undoubted homology with other tettigoniids. The external morphology of the tympana and tracheal openings was examined by scanning electron microscopy (Hitachi S-530). The internal tracheal anatomy was examined in freshly killed material. The anatomy of the legs, and particularly the arrangement of the receptors within the proximal tibia of all three pairs of legs, was examined with cobalt backfillings of the nerves. Insects were anaesthetized using CO_2 and the legs were transferred to a saline (Clements and May, 1974) bath. The tympanic or leg nerve was exposed on the ventral side of the knee and this cut end was placed in a glass capillary tube filled with $1.5 \text{mol} \, l^{-1}$ $CoCl_2$. After 24 h at 4°C the capillary was removed, the dye precipitated with 1% ammonium sulphide, and the leg was dehydrated in alcohol and then stored in styrene, which cleared the cuticle. This allowed visual examination of the stained receptors and nerves. Microphotography was made difficult by the dense pigmentation beneath the cuticle and so the fine structure of the arrangement of receptor cells associated with the leg tracheae was revealed by careful removal of the cuticle and underlying tissue. A sample of the legs was embedded in Agar 100 (Agar Aids, Stansted, UK) or paraffin wax and sectioned. The central projections of the sensory fibres of the tibial organ were investigated by anterograde labelling of the tympanic nerve with $0.2 \text{mol} \, l^{-1}$ $NiCl_2$. Anaesthetized insects were fixed ventral side uppermost and the leg was opened at the proximal femur and trochanter. The tympanic nerve was cut and placed in a capillary in the same manner as for peripheral staining. After 4 h, the dye was precipitated with a solution of rubeanic acid (Quirke and Brace, 1979) and the ganglion was silver intensified (Bacon and Altman, 1977). Some ganglia were fixed and embedded in Agar 100 for subsequent sectioning.

Neurophysiology

Adult insects of both sexes were stuck with wax, dorsal side down, to a metal holder. The connectives between the suboesophageal and prothoracic ganglia, or the leg nerve of each leg as it entered the respective thoracic ganglion, were exposed ventrally. The legs were fastened to a 10 cm rod attached to the oscillating plate of a vibrator (Bruel & Kjaer, Minishaker 4810). The position of the legs with respect to the body was as natural as possible. The legs were stimulated *via* the minishaker with a range of sinusoidal vibrations between 30 and 5000 Hz, driven by a standard sine-wave generator (Burchardt, AS II). The stimulus was provided for 100 ms, with a rise and fall time of 1 ms, and was repeated every 0.5 s. The acoustic stimulus was presented using a loudspeaker (Isophon PSM 120/8 or Audax TW 8B) ipsilateral to the preparation and in the same plane. Sound pulses
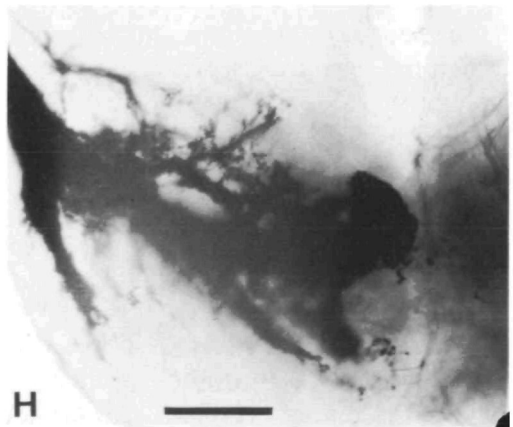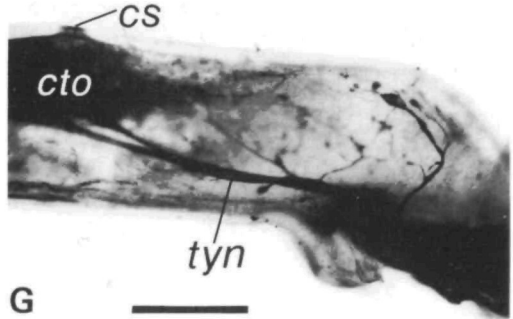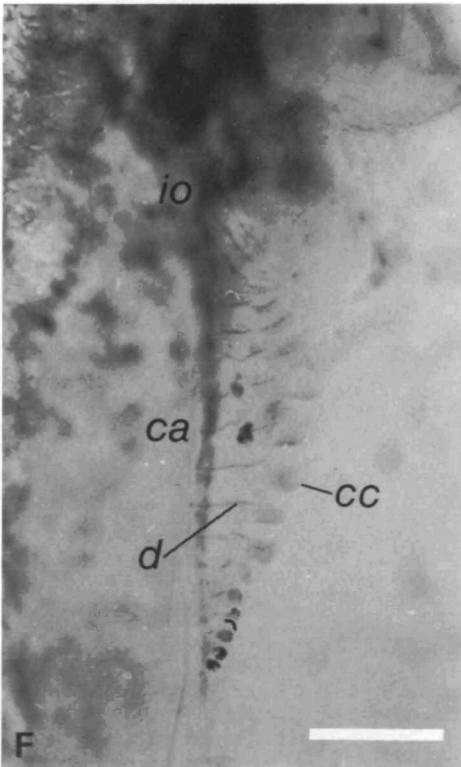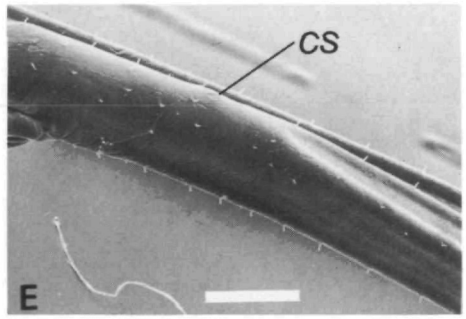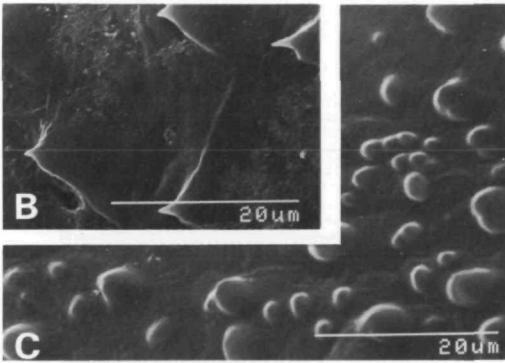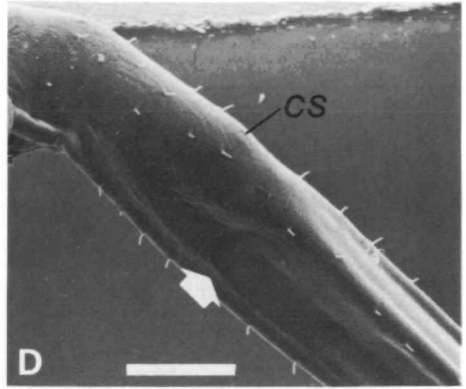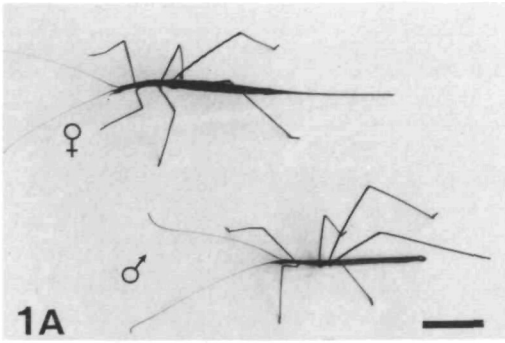
Fig. 1. *Phasmodes ranatriformes* and its auditory system. (A) Female (upper) and male (lower) *P. ranatriformes*. Scale bar, 2 cm. (B) Scanning electron micrograph of the scale-like appearance of cuticle of parts of the leg other than the region of the 'tympanum'. (C) Part of the cuticle of the tympanum of the foreleg tibia showing small protuberances. (D) Scanning electron micrograph of the foreleg tibia of a female. The broad arrow marks the area of the tympanum (*cs*, campaniform sensilla). Scale bar, 500 μm. (E) The tibia of the middle leg of the same animal, with no visible cuticular specialisations at the complex tibial organ. Scale bar, 750 μm. (F) The array of receptor cells of the crista acustica of a foreleg labelled with cobalt chloride (*ca*, crista acustica; *cc*, cap cells; *d*, dendrites; *io*, intermediate organ). Scale bar, 50 μm. (G) The course of the tympanic nerve (*tyn*) in the proximal tibia. Note the heavy pigmentation at the complex tibial organ (*cto*). Scale bar, 500 μm. (H) The central projection of the tympanic nerve in the prothoracic ganglion revealed by nickel backfilling and subsequent silver intensification; rostrad is to the top. Note the dense arborization area near the midline, which resembles the projection of tympanal receptor fibres in tympanate tettigoniids. Scale bar, 100 μm.

were derived from a sine-wave generator (Burchardt, AS II) over a range of 200–40 000 Hz with a maximum intensity of 95 dB SPL. The time parameters were as for the vibratory stimulus.

The responses of the neck connective and the nerves of the fore-, middle and hindlegs were monitored with a single steel hook electrode. The amplified signal was displayed and recorded on tape and at the same time sections were recorded on film. Post-stimulus time histograms were made by replaying 10 successive stimuli using a threshold discriminator and software (courtesy of M. Sippel) run on a Z-80 microprocessor. The response threshold was defined as the level at which no responses could be detected aurally from the monitoring loudspeaker linked to the nerve preamplifier, giving a resolution of about 2 dB SPL.

Results and Discussion

Morphology

The generalized structure of the tettigoniid hearing system

The most obvious structures of the auditory system in the forelegs of the tettigoniid are the two tympanic membranes beneath the knee. These membranes are usually formed from thin leg cuticle on the outer side and from the acoustic trachea on the inner side (Schumacher, 1973). The trachea expands beneath the knee and forms a median membranous septum at the level of the sensory cells of the crista acustica. The trachea normally leads to an opening on the prothorax, which in most tettigoniids is independent of the tracheae associated with ventilation; its function is dedicated to hearing (see Bailey, 1990, for a review). Normally the receptor cells form three discrete organs, the crista acustica itself, the subgenual organ and the intermediate organ, and provide a system responsive to airborne and substratum vibration (Schwabe, 1906; Lakes and Schikorski, 1990). Differential movements of the internal structures of this part of the tibia

caused by changing pressures on either side of the tympanic membranes are transduced to the linear array of sensory cells of the crista acustica. However, in *P. ranatriformes* this array is reduced, and associated with this is a lack of specialization of the tracheae and the absence of the tympanic membranes themselves.

The auditory trachea

The auditory trachea of *P. ranatriformes* opens into the prothoracic spiracle at the caudal end of the elongated prothoracic segment. The spiracle is closed by three valves typical of the cricket spiracle (Ander, 1939) and similar to that described for male Zaprochillinae (Bailey and Simmons, 1991). The diameter of this opening is approximately 600 μm in the female. The spiracle opens directly into six tracheae, all of which are analogous to those of other tettigoniids and can be named using the terminology of Ander (1939) (Fig. 2). There is no separation at the spiracle into two discrete openings, one for the acoustic trachea and one for the respiratory tracheae, like those formed during the postembryonic development of tympanate tettigoniids (Anders, 1939).

In *P. ranatriformes* one branch from the prothoracic spiracle forms a lateral trunk (trachea cephalica ventralis) and leads to the head, forming small anastomoses with the corresponding trunk of the other side of the body *en route*, in the region of the prothoracic ganglion (transverse anterior commissure). A branch from the lateral trunk enters the leg (trachea pedalis anterior) and splits almost immediately to supply the muscles of the coxa, trochanter and femur. The second trachea, which enters the coxa of the foreleg, is the trachea supraventralis (Fig. 2). This trachea retains its diameter within the leg and divides into two tracheal elements in the tibia. This trachea is analogous to the acoustic trachea and auditory bullae of most tympanate groups, although it originates in the prothoracic spiracle and not through a separate auditory spiracle. It has a respiratory function. Within the thorax the trachea supraventralis produces small branches along the body wall and anastomoses with the equivalent branch from its counterpart on the opposite side of the prothorax (suprafurcal connective, Fig. 2). This is homologous to the anastomosis described in both gryllacridids and crickets (Ander, 1939; Ball and Field, 1981). In most tettigoniids (Bailey, 1990) the suprafurcal connective connects with the acoustic bulla, an expansion of the trachea that acts either as an acoustic horn (Lewis, 1974; Hill and Oldfield, 1981) or as a resonator (Nocke, 1975). After entering the femur, the trachea supraventralis may function as the main leg trachea, as it gives rise to smaller branches within this segment leading to the more distal parts of the leg.

In the tibia, the main trachea expands, filling almost the whole diameter of the proximal tibia (Fig. 3). In this area the trachea has a dorsal infold, which disappears distally where the trachea is constricted at the beginning of the subgenual organ. At the proximal end of the crista acustica, the trachea bifurcates into a larger posterior trunk and a smaller anterior branch, both extending to the distal tibia. In some preparations, the anterior branch separated shortly after the
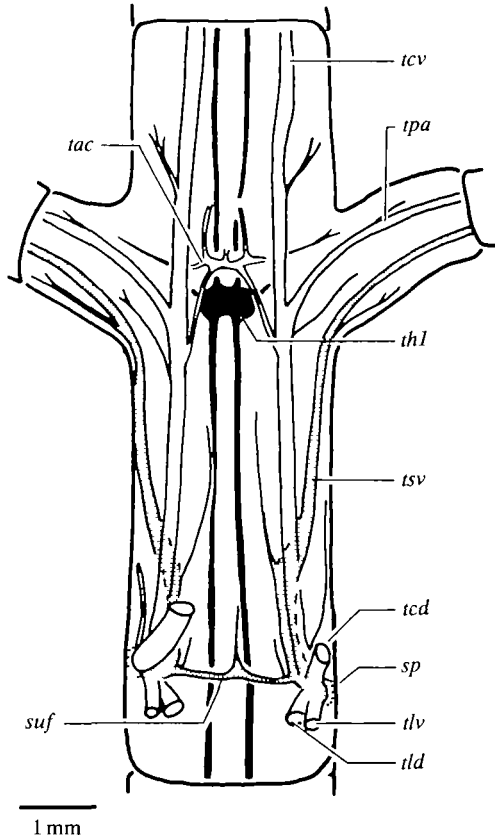
Fig. 2. The anatomy of the prothoracic tracheal system drawn after ventral dissection. Six, probably respiratory, tracheae arise from the prothoracic spiracle. The trachea that is analogous to the tympanic trachea of tympanate tettigoniids is shaded. The nomenclature follows the scheme of Ander (1939). *sp*, prothoracic spiracle; *suf*, suprafurcal connective; *tac*, transverse anterior commissure; *tcd*, trachea cephalica dorsalis; *tcv*, trachea cephalica ventralis; *th1*, prothoracic ganglion; *tld*, trachea laterodorsalis; *tlv*, trachea lateroventralis; *tpa*, trachea pedalis anterior; *tsv*, trachea supraventralis (acoustic trachea).

trachea entered the tibia and, in these cases, the tracheal expansion was only observed in the posterior branch.

The proximal tibia

Some 200 μm below the knee of the foreleg the round cross section of the leg changes into a more angular cross section, with pronounced ridges along the outer surface (Figs 1D,E, 3). The tympanic area is visible as a somewhat flattened sunken area of cuticle with a markedly different surface structure to that of the surrounding tibia (Fig. 1B,C). The cuticle forming the tympanic area is covered with small raised protuberances, 2–7 μm in diameter, in contrast to the rather more scale-like protuberances of the surrounding cuticle. No fibrillar structures,

Proximal

sn

cs

sm
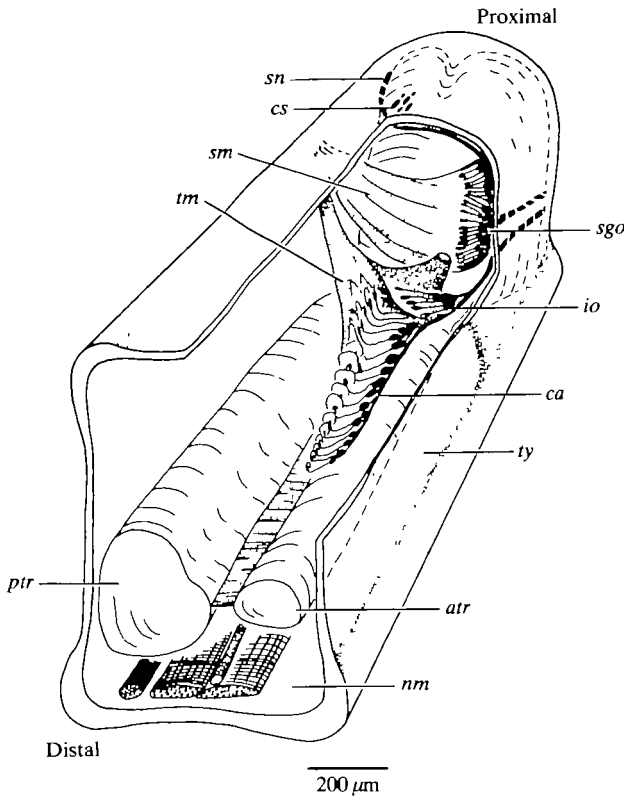
tm

sgo

io

ca

ty

ptr

atr

nm

Distal

200 μm

Fig. 3. Semi-schematic three-dimensional view of the complex tibial organ of a foreleg. The acoustic trachea narrows at the proximal tibia before it bifurcates. Three scolopidial receptor organs (the subgenual organ, the intermediate organ and the crista acustica) are arranged within the haemolymph channel above the tracheae. The crista acustica extends along the anterior trachea, accompanied by thin leg cuticle, the tympanum. *atr*, anterior trachea; *ca*, crista acustica; *cs*, campaniform sensilla; *io*, intermediate organ; *nm*, nerve muscle channel; *ptr*, posterior trachea; *sgo*, subgenual organ; *sm*, supporting membrane of the subgenual organ; *sn*, subgenual nerve; *tm*, tectorial membrane; *ty*, tympanum.

such as are found in tympanate tettigoniids (Schumacher, 1975), can be seen. The cuticle is about 5 μm thick in this region of the leg, whereas the surrounding cuticle is about 17 μm thick. Nevertheless, the cuticle of the tympanic area is substantially thicker than the tympanic membrane of most tettigoniids, where it is usually approximately 1–1.5 μm thick (Schumacher, 1978). By comparison, the middle and hindlegs show no evidence of a changed cuticular structure in the area of the crista acustica.

The tympanic receptors

The general organization of the three receptor organs within the proximal tibia of *P. ranatriformes* is the same as in most other tettigoniids (Fig. 3). Three sensory

organs can be distinguished: the subgenual organ, the intermediate organ and the crista acustica.

The subgenual organ consists of scolopidial cells with their associated attachment cells. The supporting membrane forms a curtain hanging within the haemolymph channel, as in other Ensifera. This membrane, which is attached to the laterodorsal surface of the leg, is often clearly visible because of the heavy pigmentation of the accessory cells. The scolopidial cells are arranged in parallel underneath the dorsal anterior surface of the cuticle. The tympanic nerve innervates some 18–23 sensilla in this organ compared to about 20 sensilla in other tettigoniids (Schumacher and Houtermans, 1975; Lakes and Schikorski, 1990). The second part of the subgenual organ is innervated by the subgenual nerve, a branch of the leg nerve. The intermediate organ consists of two groups of cells, both innervated by the tympanic nerve. The variable innervation is like that of tettigoniids (Lakes and Mücke, 1989). About five cells form the proximal part of the organ. This count was made from the dendrites because the cell bodies themselves were difficult to identify by light microscopy. In the distal part of the intermediate organ, the 12–13 receptor cells are stretched onto a tectorial membrane in much the same manner as in the crista acustica. It is thought that the function of the subgenual and the intermediate organs is primarily the perception of vibrational stimuli, although the receptor cells of the intermediate organ are able to perceive airborne sound in tympanate tettigoniids (Zhantiev and Korsunovskaya, 1978; Kalmring *et al.* 1978).

The crista acustica is undoubtedly the most interesting part of the sense organ array in *P. ranatriformes* as its primary function in the tettigoniids is to transduce airborne sound. The cuticle above this organ in *P. ranatriformes* is thick and does not appear to function like the tympanic membranes of other tettigoniids. In fact, the cuticle and the tracheal arrangement of the forelegs of *P. ranatriformes* is much more like the anatomy of the middle and hindlegs of other species (Friedrich, 1927; Houtermans and Schumacher, 1974; Lakes and Schikorski, 1990). The receptor cells, lying on the anterior trachea, form a row down the long axis of the leg (Figs 1F, 3). Their cell bodies and associated attachment cells become smaller towards the distal end of the crista acustica, reflecting the classical pattern of receptors for this group (Schwabe, 1906; Lakes and Schikorski, 1990). The diameter of the receptor cells ranges from 15 μm for the distal cells to 37 μm for the median receptor cells. The dendrites from these cells run above the connective tissue between both tracheal elements. The cap cells are often pigmented and easily visible, reaching their maximum size about half way along the crista acustica (Fig. 1F). The tectorial membrane, to which the cells are attached, arises at the proximal end of the organ and is linked to the dorsal cuticle of the leg. We counted 16–18 receptor cells in the array for the females (N=6) and 16–17 cells for the males (N=3). This small variation is undoubtedly due to the difficulty of staining the small distal cells and of separating the proximal cells from the cells of the intermediate organ (for a discussion, see Lakes and Schikorski, 1990).

The anatomy of the middle and hindlegs is comparable to that of the forelegs

(data not shown), although there is a reduced number of cells forming the structural equivalent to the crista acustica of the foreleg. 13–15 receptor cells were found in the middle leg (N=6) and 11–13 cells in the hindleg (N=6) in both males and females. In these legs it is even more difficult to distinguish the distal part of the intermediate organ from the crista acustica, as dendrites of both organs are attached to the same tectorial membrane and are innervated by branches of the tympanic nerve. Thus, in *P. ranatriformes*, the arrangement of the receptor arrays and tracheae in all three leg pairs appears to be very similar and is comparable to that of the middle and hindlegs of other tettigoniid species.

The tympanic membranes and the tracheal arrangement of the tibia of the foreleg of *Tettigonia viridissima* develop progressively postembryonically (Eggers, 1928). In early instar larvae the tracheae are separated; they fuse in the later stages. The cuticle of the tympanic membranes becomes thinner during development and the membranes are fully functional only in the adult animal. The sensory organ itself seems to be present in the first-instar larvae and the receptor cells arise during embryogenesis (Meier and Reichert, 1990). The anatomy of the foreleg of the adult *P. ranatriformes* is, therefore, similar to that seen in the early postembryonic development of other tettigoniids.

The innervation of the legs

The leg nerve, after it leaves the ganglion, splits off small nerves to the muscles of the coxa, whereas the main trunk divides into the leg nerve and the tympanic nerve at the coxa–trochanter joint (data not shown). Smaller branches innervate three fields of campaniform sensilla and hair plates within the trochanter, one field in the coxa and two fields in the proximal part of the femur. The leg nerve splits into sensory branches, which supply hair receptors of the femur, and motor branches. The subgenual nerve and smaller branches running to sensory hairs at the posterior leave the leg nerve in the proximal tibia. The leg nerve divides again into two trunks, which supply the muscles and the sense organs of the distal tibia and the tarsae.

The chordotonal organ of the proximal femur is divided into two parts, both of which are innervated by branches from the tympanic nerve. They contain about 25 (distal part) and 40 (proximal part) receptor cells. Without further branching in the femur, the tympanic nerve reaches the proximal tibia, where it branches in a pattern typical of other tettigoniids (Fig. 1G; Lakes and Mücke, 1989; Lakes and Schikorski, 1990). The main trunk runs to the intermediate organ and the crista acustica, sending a proximal branch to the subgenual organ. Additional branches supply receptors (hair sensilla, campaniform sensilla) on the cuticle of the dorso-anterior part of the proximal tibia. No branches proceed beyond the group of sense organs.

Central projections

Centripetal labelling of the tympanic nerve shows sensory projections comparable to the pattern known for the different receptors in other insects. The
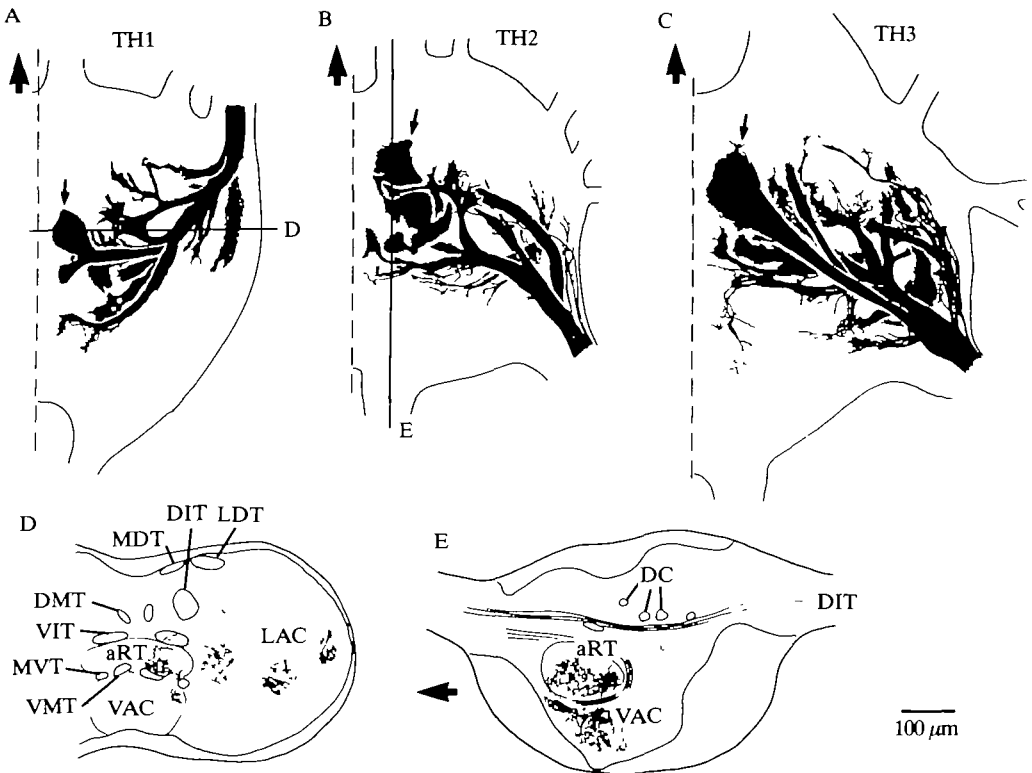
Fig. 4. Central projection of the tympanic nerve in the ventral nerve cord as revealed with nickel backfills. (A) Prothoracic ganglion (TH1); (B) mesothoracic ganglion (TH2); (C) metathoracic ganglion (TH3); (D) transverse section indicated in A. (E) Parasaggital section of the mesothoracic ganglion indicated in B. aRT, anterior ring tract; DC, dorsal commissures; DIT, dorsal intermediate tract; DMT, dorsal median tract; LAC, lateral association centre; LDT, lateral dorsal tract; MDT, median dorsal tract; MVT, median ventral tract; VAC, ventral association centre; VIT, ventral intermediate tract; VMT, ventral median tract. The arrows in A–C point to a dense neuropile near the midline, which is probably formed by the projection of the fibres of the complex tibial organ. Projections in the lateral areas are likely to originate from hair receptors and campaniform sensilla. Anterior is indicated by the broad arrows.

tympanic nerve joins the leg nerve, which enters the prothoracic ganglion rostrad and the meso- and metathoracic ganglia caudad. Labelling of the whole nerve allows a description of the projections of all receptor fibres of the tympanic nerve, but makes it difficult to distinguish the structure of individual receptors (Figs 1H, 4). However, major arborization areas can be recognized and compared with the central projections in other tettigoniids and acridid grasshoppers.

The general neuroanatomy of the fibre tracts and the neuropile areas of the thoracic ganglia seems to be common to all tettigoniids (Lakes and Schikorski, 990), including *P. ranatriformes*. Three major projection areas are recognizable:

in the lateral, the ventral and the median neuropiles. The most interesting projection area is the medial ventral association centre (Pflüger *et al.* 1988) or anterior ring tract (aRT, Tyrer and Gregory, 1982). Auditory afferents and fibres of the proximal chordotonal organ of the femur end, in tympanate tettigoniids, within that neuropile (Römer, 1983; Römer *et al.* 1988; Pflüger *et al.* 1988). In *P. ranatriformes*, similar dense neuropile is formed near the median midline (Fig. 4) and sections show that afferents, presumably auditory fibres, end in the aRT. The aRT of the prothoracic ganglion is not enlarged like that of other tettigoniids (Lakes and Schikorski, 1990). Consequently, compared with those in the meso- and metathoracic ganglia, the projection area of the fibres is rather small. The projections are, however, comparable to those in the meso- and metathoracic ganglia of tympanate tettigoniids, where no tympanal organ is present in the middle and hindlegs. Nevertheless, we presume that the fibres of the crista acustica end within the segmentally homologous aRT (Lakes and Schikorski, 1990). In other words, the staining seen in the aRT of *P. ranatriformes* originates, at least partly, from fibres of the crista acustica of the complex tibial organ.

Similarly, other projections in *P. ranatriformes* can be ascribed to certain types of sensilla by comparison with known projections of identified receptors in other Orthoptera. For example, fibres terminating within the lateral association centre (Pflüger *et al.* 1988) are likely to originate from campaniform sensilla and hair plates (Pflüger *et al.* 1988). Projections into the ventral association centre (Fig. 4) are probably formed from fibres of hair sensilla.

Neurophysiology

Recordings from the leg nerve

Responses to both airborne and substratum-borne signals were recorded from the leg nerves of the pro-, meso- and metathoracic legs as they enter the respective ganglia. In the foreleg, a response to pure-tone vibrations up to 5000 Hz was found with a maximum sensitivity in the range 200–800 Hz (Fig. 5). The threshold acceleration, measured by the first detectable response, was $0.005\,\mathrm{m\,s^{-2}}$. Sensitivity in the middle leg was slightly lower, with a threshold acceleration of $0.009\,\mathrm{m\,s^{-2}}$. The most sensitive leg was the much longer hindleg, where threshold values of $0.002\,\mathrm{m\,s^{-2}}$ were recorded (Fig. 5) (Autrum, 1941; Kalmring *et al.* 1978; Kühne, 1982; Schikorski and Lakes, 1988). The threshold of single units in the middle leg of *Decticus albifrons* can be as low as $0.001\,\mathrm{m\,s^{-2}}$ and in this species there are only small differences in threshold values for the different legs (Kühne, 1982). In crickets, the most sensitive leg is the hindleg (Dambach, 1972), a situation comparable to that in *P. ranatriformes*.

The foreleg was the only leg to provide any measurable and repeatable response to airborne sound, but the threshold levels were far above values obtained for tympanate tettigoniids, which range from 25 to 40 dB SPL (Kalmring *et al.* 1978; Fig. 5). Maximum responses were within a wide frequency range of 400–5000 Hz and to intensities above 70 dB SPL. There was no clear frequency maximum an
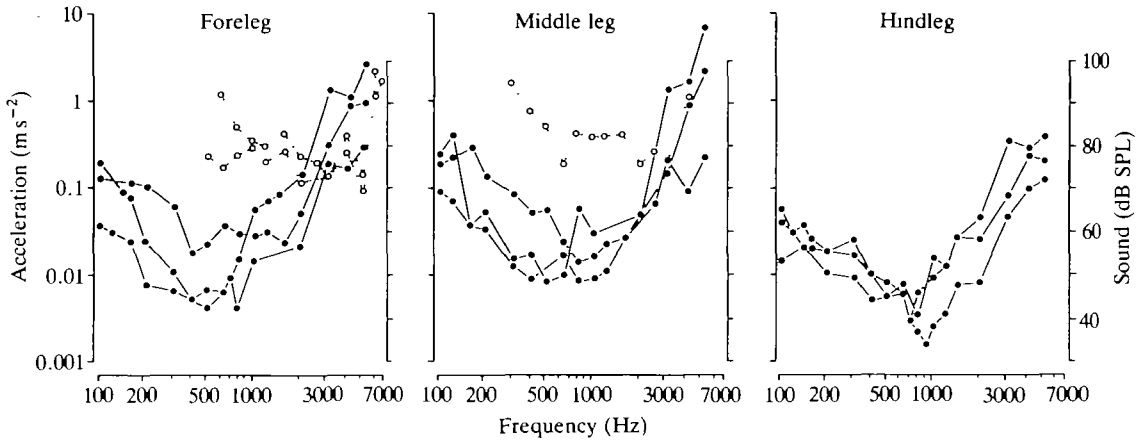
Fig. 5. Threshold curves for the response of the leg nerves of different animals to vibrational (●) and acoustic (○) stimuli. The responses were recorded extracellularly with hook electrodes. Responses to airborne sound could be recorded repeatedly from the leg nerve of the foreleg, but were recorded once only from the middle leg. The hindleg appears to be most sensitive to vibrational stimuli. The threshold values are comparable to those found in other tettigoniids.

above 5000 Hz the roll-off was rapid. Despite numerous trials (*N*=13; eight insects), we were only able to achieve a recognizable response to airborne sound from one middle leg. On this single occasion the frequency range of the response was similar to that of the foreleg and at similar threshold values. This implies that the contribution of the 'vestigial' tympanic membrane on the foreleg to the detection of airborne sound is no more than that of the cuticle covering the crista acustica on the middle leg. We attribute the improved responsiveness, but not threshold, of the foreleg to the larger number of receptors in its crista acustica sensory field. We tested sounds up to 40 kHz, but there was no indication of a response to these ultrasonic frequencies.

Recording from the ventral nerve connective

In tettigoniids, information from the auditory–vibratory receptors of the three pairs of legs is transmitted *via* uni- or bimodal interneurones to the brain (Kalmring and Kühne, 1980). The extracellular recordings from the connectives of *P. ranatriformes* show characteristic discharge patterns for different units. First, a unit with large spikes responds in a phasic–tonic manner to vibrational stimuli (see Fig. 7). Second, a tonic response occurs, which is more irregular and which begins some 20 ms after the phasic response. This second unit shows habituation to repeated stimuli.

When both forelegs were mounted on the minishaker, we noted a threshold of $0.008 \, \mathrm{m \, s^{-2}}$ in the most sensitive frequency range of 600–800 Hz (Fig. 6A). This threshold corresponds to that measured from the tympanic nerve, but the tuning was sharper. The higher-frequency response observed in the leg nerve was missing
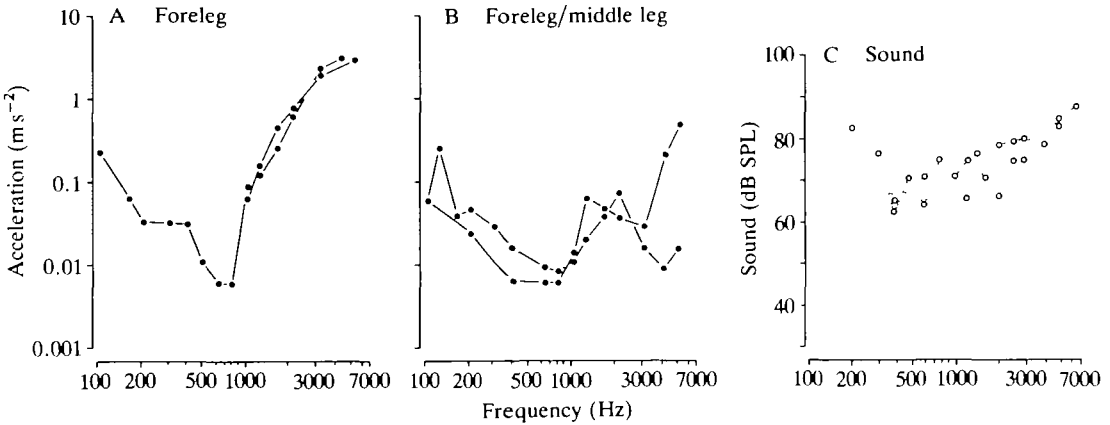
Fig. 6. Threshold curves for neurones of the neck connective of different animals recorded extracellularly. (A) Vibrational stimulation of the foreleg alone. (B) Simultaneous vibrational stimulation of the fore- and middle legs. (C) Response to airborne sound (stimulation of all legs).

in the ventral nerve cord and the roll-off in response to high frequencies was steeper. More remarkable was the difference that occurred if both the forelegs and middle legs were attached to the minishaker and stimulated with the same signal. The sharp tuning obtained with the forelegs alone was lost and a second peak appeared between 2000 and 4000 Hz with a threshold at $0.01\,\mathrm{m\,s^{-2}}$ (Fig. 6B). This implies that the middle legs are providing additional high-frequency information, augmenting the response of the forelegs. The recruited units, however, may be different when the forelegs alone are stimulated and when both forelegs and middle legs are stimulated. For a detailed analysis of the physiology and anatomy of these neurones, experiments using intracellular recordings must be carried out. As with the leg nerves, the responses of the neck connective to airborne sound show a very high threshold at all frequencies (Fig. 6C).

Latencies of the whole nerve to vibration are 18–21 ms for high intensities, and increase to 32–35 ms at lower vibration intensities (Fig. 7). Latency may extend to 45 ms near threshold, when either the foreleg is stimulated alone or both middle and hindlegs are stimulated together. Latencies to airborne sound were longer and much more variable, especially at intensities near threshold. When stimulated at 85 dB SPL, the latency was about 37 ms (Fig. 7). This compares with 15 ms for suprathreshold stimuli in *Tettigonia viridissima* (Rheinlaender and Römer, 1980). In tympanate tettigoniids, a large number of interneurones sensitive to vibration and/or auditory stimuli have been described (Kalmring and Kühne, 1980). Although we cannot prove any homology with the neurones recorded from *P. ranatriformes*, the characteristics of the first unit (large spikes, phasic-tonic response pattern) match those of a large auditory T-fibre in tympanate tettigoniids (Suga, 1963; McKay, 1969; Kalmring *et al.* 1979) that is identifiable with hook electrodes. In tympanate tettigoniids, the fibre, called the S1 neurone, shows no supra
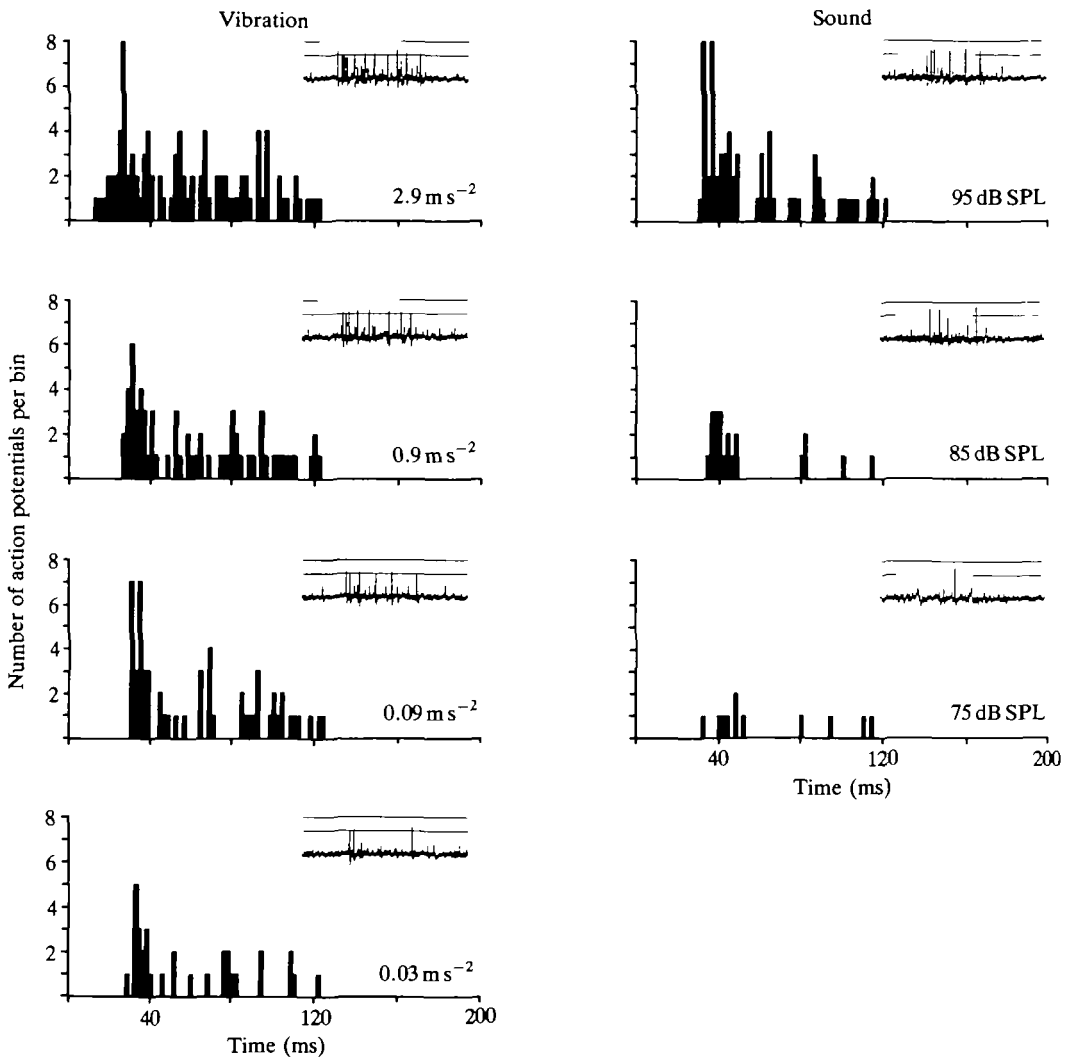
Fig. 7. Poststimulus time histograms and original recordings of the response of the neck connective of a male animal to vibrational (1000 Hz; left-hand column) and acoustic (2000 Hz; right-hand column) stimuli at different intensities. The forelegs and the middle legs were attached to the minishaker. Bin width was 2 ms; 10 consecutive stimuli were analysed.

threshold responses to vibrational stimuli. Other neurones that have been described in tympanate tettigoniids show different response patterns to vibration stimuli (Kalmring and Kühne, 1980) and may be homologous to the tonic responding unit of *P. ranatriformes*.

The evolution of hearing loss

The auditory system of tettigoniids has evolved both in a social context and as a

warning system to detect advancing predators. Bailey (1990), in describing the comparative anatomy of the hearing system of tettigoniids, points out an association between extreme crypsis and a reduction in one of the two auditory inputs to the hearing system in phytophagous insects. The implication is that crypsis may have replaced the requirement for an acoustic defence system. Certainly, in many leaf-like Pseudophyllinae, where crypsis is extreme, the normally large tracheal opening of the auditory bulla is closed; yet social signalling still depends on auditory cues. Where sound production is reduced, as in many neotropical species (Morris *et al.* 1989), there does not appear to be a concomitant reduction in hearing sensitivity, and it may be that the predator detector function is sufficiently efficient under these conditions. In some species of tettigoniids, acoustic communication is reduced to substratum vibration. However, as in *Meconema thalassinum*, the main characteristics of the tympanum of the foreleg are unchanged, except that the number of receptor cells in the crista acustica is reduced (Schumacher, 1975, 1979) and the tracheal opening is small (Ander, 1939). *Phasmodes ranatriformes* may be an extreme of this evolutionary line. The tympana do not show the extreme thinning characteristic of an acoustically sensitive membrane and the number of cells in the crista acustica is reduced. In addition, there is no development of an internal auditory trachea. With its cryptic body shape and its loss of sound-producing structures, *P. ranatriformes* may well have taken the same evolutionary route as many grylline crickets, which, with the loss of sound production, have either reduced or lost one, or both, tympana (Otte and Alexander, 1983).

Does *P. ranatriformes* reflect an extreme form of hearing reduction or has it never developed such a communication system? Unfortunately, the occurrence of such a reduced hearing system in a monotypic subfamily does not allow us to speculate by comparing related species. However, if we look at the development of the auditory system of other tettigoniids, we find that the anatomy of the auditory system in the nymphal stages (Eggers, 1928) resembles that of the adult system of *P. ranatriformes*. The crista acustica may not represent a specialized structure dedicated to the detection of airborne sound, and it is also present in the atympanate middle and hindlegs. It appears that all embryonic orthopterans develop a row of sensory cells distal to the subgenual organ in the legs (R. Lakes-Harlan, unpublished results; T. Meier, personal communication). Such an organ could be the common ancestor of the auditory system of tettigoniids, as it is found in a wide range of insects and is referred to as the distal organ (Debaisieux, 1938). Its hypothetical function in orthopterans is to measure the haemolymph pressure. Although a homology to the crista acustica has not as yet been established, these data from embryonic development suggest that the situation in *P. ranatriformes* is primitive, and indicate the origin of the hearing system of tettigoniids.

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