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FUNCTIONAL CONSEQUENCES OF A NOVEL MIDDLE EAR ADAPTATION IN THE CENTRAL AFRICAN FROG *PETROPEDETES PARKERI* (RANIDAE)

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Summary

During the breeding season, each tympanic membrane of males of the Old World treefrog *Petropedetes parkeri* is decorated with a single, prominent, fleshy tympanic papilla. The tympanic papilla, located dorsally on the tympanic membrane, is covered by an epidermal surface and is composed of non-ossified, spongiform tissue containing a number of globular, fluid-filled vesicles found at highest density near the papillar tip. These vesicles appear to have exit pores and are probably simple alveolar exocrine glands. Injecting sound into the pressurized vocal cavity of the male and measuring the vibration velocity response of the tympanic membrane revealed that from 0.3 to 2.0 kHz the tympanic papilla velocity amplitude is on average 20 dB lower than that of a point diametrically opposite on the ventral half of the tympanic membrane. The close agreement between the dominant frequency of the call and the frequency of the maximum spectral peak of the Fast Fourier Transform of the impulse response of the eardrum is consistent with the use of the eardrum in this species both as a call receiver and as a call radiator, similar to the function suggested for the eardrum of the male bullfrog *Rana catesbeiana*. Unexpectedly, surgically removing the tympanic papilla lowered the frequency of the peak vibrational amplitude, testifying to the importance of membrane tension as a dominant factor in the vibratory behavior of the eardrum. During normal positive-pressure breathing, the tympanic papillae move conspicuously, suggesting a possible rôle as a visual signal.

Key words: tympanic membrane, Ranidae, Cameroon, *Petropedetes parkeri*, columella, tympanic papilla, communication.

Introduction

Male anuran amphibians (frogs and toads) use auditory (Bogert, 1960; Littlejohn, 1977; Wells, 1977; Gerhardt and Schwartz, 1995) and visual (for a review, see Hödl and Amézquita, 2001) cues to attract conspecific females and to defend territories against potential conspecific intruders. In most species of anuran, males are vocal and females are not, representing a clear example of a behavioral difference between the sexes. Males and females may also exhibit external morphological and other differences. For example, secondary sexual characteristics are traits other than those associated with the gonads and their ducts that differ between the sexes (Noble, 1931). The development of secondary sexual characteristics, resulting in sexual dimorphism, is a common phenomenon among the anurans (duToit, 1943). These characteristics may persist throughout the adult life of the animal; for example, the sexually dimorphic size of the tympanic membrane in some anuran species, such as the North American bullfrog *Rana catesbeiana*, in which the tympanic membrane of the male is larger than that of the female (Shofner and Feng, 1981; Hetherington, 1994; Purgue, 1997). Other examples of permanent secondary sexual characteristics are the bright coloration of males of *Bufo periglenes* compared with the females (Savage, 1967) and the unique frontal swellings on the head of male *Rana pileata* (Noble, 1931). In contrast, some species have ephemeral secondary sexual characteristics that appear only during the breeding season, in which case their expression is presumably under hormonal control. These include the nuptial pads of many male frogs and toads (Klemens, 1997), the prepollical spines on males of *Hyla rosenbergi* (Kluge, 1981) and the labial spines of

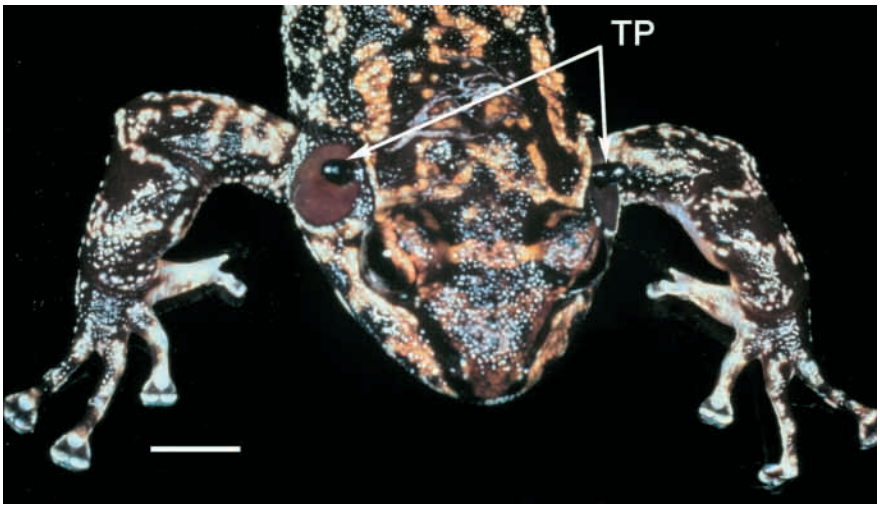

Fig. 1. The head of a breeding male *Petropedetes parkeri* showing the prominent tympanic papillae (TP) decorating each eardrum. The papillae are non-ossified spongiform structures that appear only on males and only during the breeding season. Scale bar is 5 mm.

Leptobrachium (*Vibrissaphora*) *liui* from western China (Huang, 1990).

Frogs in the genus *Petropedetes* (family Ranidae) are restricted to sub-Saharan central Africa and possibly southwest Uganda (Duellman and Trueb, 1986; Drewes and Vindum, 1994). Some *Petropedetes* males are unique in possessing a seasonal secondary sexual characteristic in the form of a tympanic papilla (duToit, 1943; Lawson, 1993; R. C. Drewes, personal communication). Robust tympanic papillae are known to decorate male eardrums of four out of seven known species of adult *Petropedetes*: *P. cameronensis*, *P. johnstoni*, *P. newtoni* and *P. parkeri* (Perret, 1966; Amiet, 1983). There has been considerable confusion in the literature regarding the structure of this papilla, and no previous explanation has been provided for its function. For example, *Petropedetes newtoni* has been described as having a columellar process 'thrust through the drum' (Noble, 1931), a columella 'protruding through the tympanum' (Duellman and Trueb, 1986) and, more recently, in *P. natator* '…ragt das Gehörknöchelchen über das Trommelfell hinaus' ('…the columella projects out of the eardrum') (Walkowiak, 1998). An alternative explanation was offered first by duToit (duToit, 1943), who demonstrated that epidermal and cuticular components formed the superficial layers of the tympanic papilla of *P. johnstoni*, whereas its internal structure was composed chiefly of collagenous fibers. We carried out a combined morphological, biomechanical and behavioral study to characterize the structure of the tympanic papilla in *P. parkeri*, one of the largest (mean male snout–vent length 55.1 mm; Amiet, 1983) of the seven known species of *Petropedetes*, and its possible relationship to courtship behavior.

Materials and methods

Histological analysis

Tympanic papillae were prepared histologically, sectioned either parallel or perpendicular to the longitudinal axis and stained with Toluidine Blue and osmium. Slides were viewed with a microscope (Olympus BX 60) equipped with a digital camera (Camera-Spot, Diagnostic Instruments, Inc.) attached to a computer (Power Mac G-3). Images were captured using Adobe Photoshop and printed at 300 dpi (Epson Stylus Photo 700 or Tektronix Phaser 440).

Scanning electron microscopy and X-ray images

The morphology and internal structure of the tympanic papilla were studied using scanning electron microscopy, following the technique of Cristobal et al. (Cristobal et al., 1998). The tympanic papilla was sectioned longitudinally and immersed in 2 % paraformaldehyde and 2 % glutaraldehyde in phosphate buffer for 60 min. The sections were rinsed in 0.1 mol l^{-1} phosphate buffer then incubated for 2 h in 1 % osmium. The preparations were rinsed twice in fresh phosphate buffer. The papillae were then dehydrated by successive 4 min incubations in a graded alcohol series at 70 %, 80 %, 90 % and 100 %, followed by critical-point-drying with hexamethyldisilazane (Polysciences, Warrington, CA, USA). The papillae were then sputter-coated with gold and imaged with a scanning electron microscope (Hitachi, S-2460N, Hitachi Ltd, Tokyo, Japan).

X-ray photographs were prepared using an intra-oral X-ray unit (Siemens, Sirona Heliodent DS-60), operating at 7 mA and 60 kV d.c. The exposure time was 0.2 s. Images were processed with a Digital X-ray software package (Sidexis XW).

Study site and field recordings

During July 1996, we studied *Petropedetes parkeri* (Fig. 1), a close relative of *P. newtoni* (Perret, 1966; Amiet, 1983), in middle-elevation rain forest (above approximately 400 m) in the Nkwende Hills near Nguti, Cameroon (5°19.9′N, 9°24.8′E). *Petropedetes parkeri* is found on nearly vertical rock faces, along the roadsides, that are continually bathed in dripping water. Tadpoles of this species occupy an unusual niche in the semiterrestrial tadpole guild (*sensu* Altig and Johnstone, 1989) in that they are adapted to grazing vertically on algae-covered rocks (for other species that exhibit this

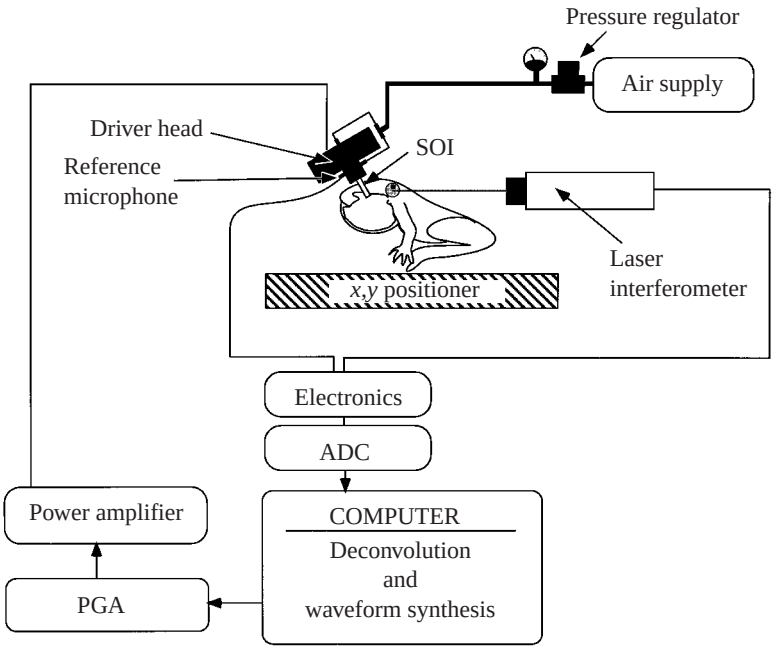

Fig. 2. Schematic diagram of the apparatus used to measure the transfer function of eardrum vibration velocity of *Petropedetes parkeri*. The standard orbital interface (SOI) is inserted into one eye orbit, through which the buccal cavity is inflated to the approximate volume observed during calling. Computer-generated pure tones are fed through the programmable gain amplifier (PGA) and power amplifier before being injected into the buccal cavity through the SOI. The resulting tympanic membrane velocity is measured with the laser interferometer oriented normal to the tympanic membrane. Both the acoustic output of the driver and the electrical output from the laser are digitized and stored in the computer. ADC, analog-to-digital converter.

behavior, see Wassersug and Heyer, 1983; Wassersug and Heyer, 1988; Altig and Johnstone, 1989; McDiarmid and Altig, 1999). Adult males of *P. parkeri* vocalize from purchases in the rocks from approximately 20:00 h to 23:00 h and can easily be localized by their calls.

The advertisement calls of six males of this species were recorded from 3 July to 16 July 1996 in their natural habitat and analyzed for this study. Two minutes of spontaneous vocalizations was registered with a directional microphone (Audio-Technica AT-835 or AKG CK8) placed 30–100 cm from each calling male and connected to a digital recorder (Sony TCD-D10 Pro II DAT). All recordings were made using low-noise digital audio tape (Sony DT-120). Temperature and relative humidity were registered after each recording using a sling psychrometer (Bachrach).

Advertisement call analysis

The spectral and temporal characteristics of a series of advertisement calls from each male were analyzed either on a DSP Sonagraph (Kay Co. 5500 Workstation) using a transform size of 100 points (filter bandwidth of 300 Hz), with a temporal resolution of 1.6 ms and a spectral resolution of 40 Hz, or with a custom-designed program for calculating either the Fourier transform (2048 points at a sampling rate of 22 kHz) or the average power spectral density function (PSDF) of a signal (A. P. Purgue, unpublished information). A complete suite of call characteristics obtained from multiple vocalizations for each of 18 males will be analyzed in a detailed companion study of the courtship behavior of *P. parkeri* (L. R. Minter, P. J. Bishop, P. M. Narins, E. R. Lewis and D. P. Lawson, in preparation).

Sound injection and pressure control in the oral cavity

To measure the response of the eardrum of a frog to its own call, we used a variation of the method of Purgue (Purgue, 1997) in which a pure tone synthesized in the computer (Power Macintosh 7100/80) was fed to a power horn driver (Radio Shack 40-1307) (Fig. 2). We used custom-designed software to generate continuous sine waves from 80 to 2500 Hz at 20 Hz intervals. Sound produced by the driver was fed through a programmable gain amplifier and power amplifier (PA 25, Radio Shack) before being injected into the oral cavity through a custom-made brass coupler connected to a short (15 mm) piece of tapered plastic tube (standard orbital interface, or SOI). The SOI was fitted and superglued (Locktite) into the left orbit of a freshly pithed specimen. The coupler was also equipped with (i) a reference microphone (Kobitone LM-046), to allow monitoring of the signal source level, and (ii) an air port through which changes in the oral cavity pressure could be introduced and monitored (Fig. 2). During measurements of the velocity of the tympanic membrane, the buccal cavity was inflated to a gauge pressure of 300 Pa. This value was chosen on the basis of recordings of the intraoral pressure during ventilation in this species (data not shown) and of recordings of similar values for other species for which pressure values during vocalization were available (Purgue, 1995; Purgue, 1997).

A small metal pipe (internal diameter 3 mm) was used to connect the front and back of the power horn diaphragm for pressure equalization during testing. The whole assembly was connected to a supply of regulated compressed air. In this way, the oral cavity could be pressurized without deforming the power horn diaphragm (Purgue, 1997).

Laser vibrometric measurements of the tympanic membrane

Prior to making the tympanic membrane velocity measurements, the preparation was mounted on a custom-made platform attached to a three-dimensional micromanipulator (Purgue and Narins, 2000). This assembly and the power horn

driver unit were mounted on a two-axis goniometric cradle (Newport M-UBG50 and M-UBG80) which, in turn, was fixed to an *x*,*y* translation platform (Newport 406). This whole assembly, together with the microscope (Zeiss OP1) and the laser head (OFV 303), was mounted on a vibration isolation table (Newport RS 4000 with four Newport I-2000 laminar flow isolators).

Because the reflectivities of the tympanic membrane and tympanic papilla are normally insufficient to provide an adequate signal for the laser vibrometer, one or more glass beads (30 μm diameter, Polytec) were placed on these structures. To obtain the velocity readings, the laser beam was then trained on each of these beads in succession. We assume that the velocities of the tympanic membrane and tympanic papilla can be accurately described by measuring the velocity of the glass beads attached to their surfaces (Purgue and Narins, 2000). The validity of this assumption depends on two conditions: (i) that the beads remain bonded to the surface of the structures being measured, and (ii) that the beads do not significantly load these structures. To meet the first condition, once the bead had been placed on the tympanic membrane or tympanic papilla, the integrity of the bond between the beads and the membrane was tested by rotating the preparation using the goniometric cradle in such a way that the surface of the tympanic membrane formed an angle of 45 ° with the horizontal. For the bead to stay in place, the resulting small gravitational component acting on the bead must be overcome by the adhesion forces between the membrane and the bead. To test for the second condition, we placed a single bead on the membrane and recorded the velocity spectrum for that bead location. Up to three additional beads were then added to the membrane in close proximity to the first bead without producing any measurable alteration in the velocity spectrum of the first bead (Purgue and Narins, 2000). Assuming a bead density of 2200 $kg\,m^{-3}$ and a mean radius of 30 μm, the mass of one bead is 2.48×10^{-7} g and the measured mass of the tympanic membrane excluding the papilla is 6×10^{-3} g. Thus, the mass load produced by the beads was considered to be negligible. The sensitivity of the laser was fixed at 1 $mm\,s^{-1}\,V^{-1}$.

Velocity amplitude responses of the tympanic membranes were measured using a laser Doppler vibrometer (sensor head, OFV 303; controller, OFV 3001; Polytec). The laser output signal was attenuated (Hewlett-Packard 350D), sampled by the analog-to-digital board in the Power Macintosh (7100/80) and displayed on the monitor screen as the relative velocity amplitude (in dB) as a function of frequency.

Impulse response measurements

To measure the natural vibration frequency of the tympanic membrane in response to a mechanical deformation at a discrete point, we used an insect pin tipped with dental cement and carefully placed at a point near the center of the tympanic membrane. The pin was slowly retracted until the adhesive forces between the dental cement and the tympanic membrane were overcome by the restorative forces acting on the tympanic membrane, resulting in the membrane 'snapping back' and vibrating at its natural frequency. The membrane velocity during this vibration was measured with the laser trained on a point near the center of the tympanic membrane.

Results

Morphology

The tympanic papilla in this species is a prominent, darkly-colored, fleshy protuberance (Figs 1, 3) that appears only on males and only during the breeding season (duToit, 1943; Lawson, 1993; R. C. Drewes, personal communication). Fig. 3A shows the off-center location of the papilla on the tympanic membrane; the papilla occupies approximately one-twelfth of the area of the tympanic membrane. A side view of the tympanic papilla is shown in Fig. 3B. In this particular specimen, the mass of the tympanic papilla was approximately 5.0 mg, its base measured 1.67 mm in diameter and its length external to the tympanic membrane was 3.16 mm.

By surgically removing the tympanic membrane from the surrounding tympanic ring, we were able to confirm that approximately three-quarters of the tympanic papilla mass resides on the outer surface of the tympanic membrane. A smaller, but conspicuous, mass of tissue lies directly in line with the papilla on the internal surface of the tympanic membrane and has a fibrous connection with the roof of the tympanic chamber. Moreover, it is clear that the tympanic papilla is not collinear with the extracolumellar cartilage; i.e. the extracolumellar cartilage contacts the inner surface of the tympanic membrane near its center while the tympanic papilla is clearly located dorsal to this (Fig. 3A). In the specimens for which we obtained eardrum measurements, the mean tympanic membrane diameter for the males was 6.85±0.68 mm (N=5) and that for the females was 5.04±0.38 mm (N=2) (means ± S.D.).

An X-ray photograph of the frog's head (Fig. 4A) and a scanning electron micrograph of a longitudinal section through the tympanic papilla (Fig. 4B) reveal that the tympanic papilla consists of non-ossified, spongiform tissue surrounded by a cuticular surface. In a live specimen, the tympanic papilla is filled with a lymph-like, colorless fluid. In addition, the tympanic papilla contains a number of hollow, globular chambers located superficially around the central core and found in highest density near the papillar tip. These resemble mucous glands in that they are rounded in form and have a short neck (Ecker, 1889).

Histology

Fig. 5a shows a portion of a longitudinal section near the tip of the tympanic papilla. Several features characterize this region of the tympanic papilla. The core is made up of a more-or-less homogeneous spongiform matrix distinguished by its lack of cellular diversity. Located around the periphery are chambers or vesicles lined with epithelial cells that appear, in some cases, to exhibit a rugose luminal surface (Fig. 5b). These appear to be simple, multicellular exocrine glands, alveolar in shape. These glands communicate with the papillar

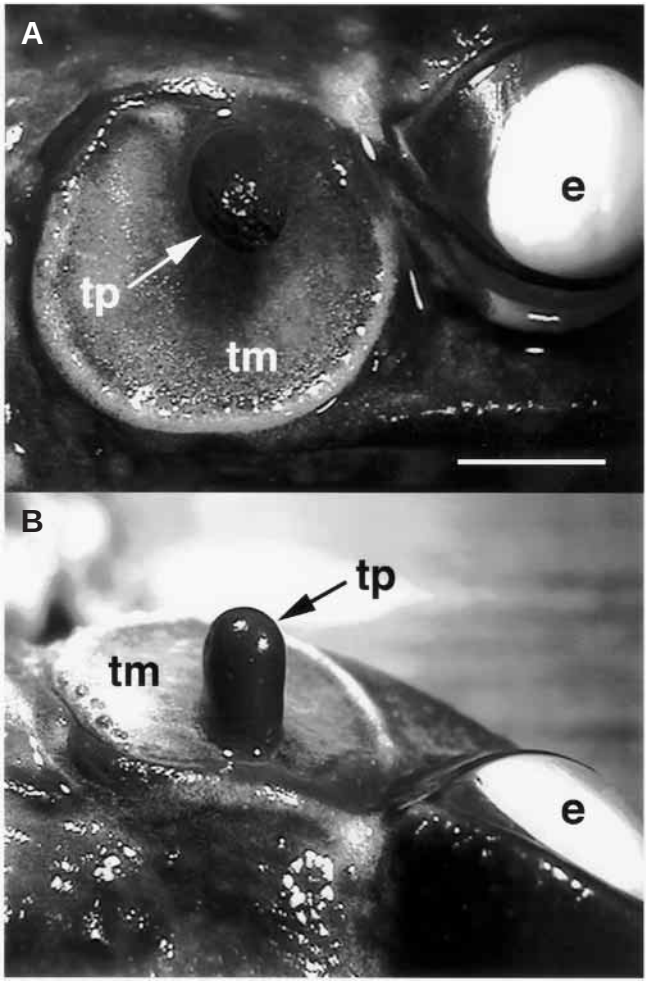

Fig. 3. (A) Lateral view normal to the tympanic membrane (tm) of the head region of a breeding male *Petropedetes parkeri*. In this species, the tympanic papilla (tp) is located off the center of the tympanic membrane and occupies approximately 12 % of the area of the tympanic membrane. (B) Dorsal view of the tympanic papilla. This papilla has a basal diameter of 1.67 mm and a length of 3.16 mm. e, eyeball. Scale bar, 2 mm.

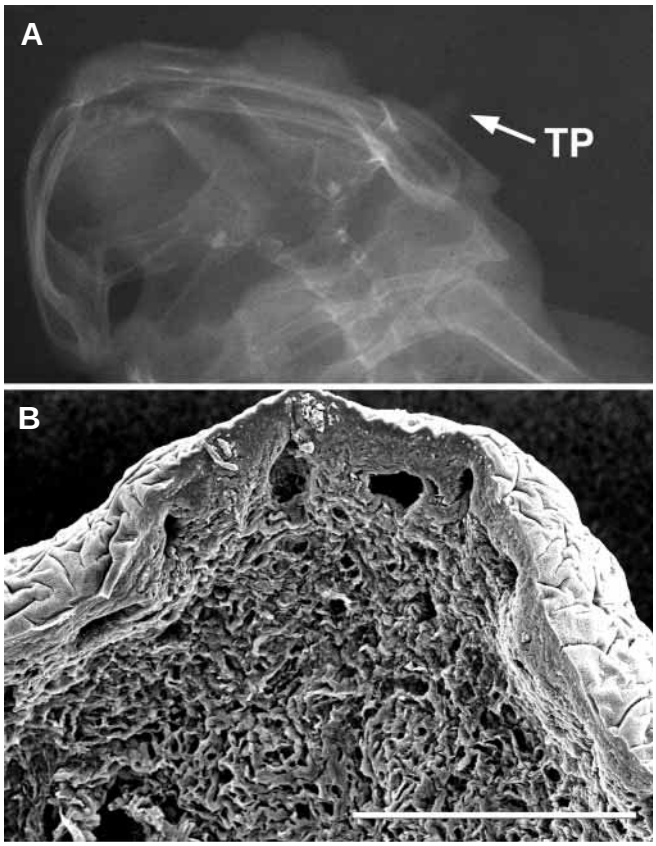

Fig. 4. (A) X-ray photograph of a ventral view of the head of a male *Petropedetes parkeri*. Note the complete lack of ossification of the tympanic papilla (TP, arrowed). (B) Scanning electron micrograph of a longitudinal section through the tip of the tympanic papilla. Note the internal spongiform tissue surrounded by the cuticular surface. Located around the periphery are chambers or vesicles that appear to be simple exocrine glands, alveolar in shape. Scale bar, 0.2 mm.

surface through a duct terminated by an exit pore that often appears to be plugged in the histological sections (e.g. Fig. 5b). Near the base of the tympanic papilla, the chambers are nearly surrounded by thick connective tissue with a wavy appearance (Fig. 5d). A subsurface layer of pigmented cells, presumably melanophores, was a prominent feature of the tympanic papilla. Located in the most superficial layer of this structure, several goblet cells were identified (Fig. 5c,e,f). These presumably have a secretory function. Control sections taken from the skin of the forearms and rear leg revealed similar goblet cells in the epidermis (data not shown). We found no evidence of cartilage or any other osteological elements in the tympanic papilla.

Advertisement call

Vocalizations are produced by males from approximately 20:00 h until shortly before midnight. The advertisement call of this species consists of a single, short (<25 ms) atonal chirp note composed of eight prominent harmonics having a fundamental frequency of 350 Hz and a dominant frequency of 1050 Hz (Fig. 6). The call is given at relatively low intensities: e.g. the dominant frequency is typically broadcast at 68 dB SPL at 50 cm (Narins et al., 1997; L. R. Minter, P. J. Bishop, P. M. Narins, E. R. Lewis and D. P. Lawson, in preparation).

Vibration velocity measurements

In response to constant-amplitude, pure-tone sweeps, the velocity responses of the tympanic papilla tip (Fig. 7, point a) and then that of a point on the eardrum diametrically opposite to the location of the papilla (Fig. 7, point b) were measured with the laser Doppler vibrometer under two experimental conditions. In the first condition, points a and b were measured in the intact ear, and in the second condition, point b was measured following surgical transection of the tympanic papilla near its base. In six frogs, one tympanic papilla was surgically excised with a scalpel by making a transverse cut as close to the surface of the tympanic membrane as possible. Over the range of frequencies between 0.3 and 2.0 kHz, the

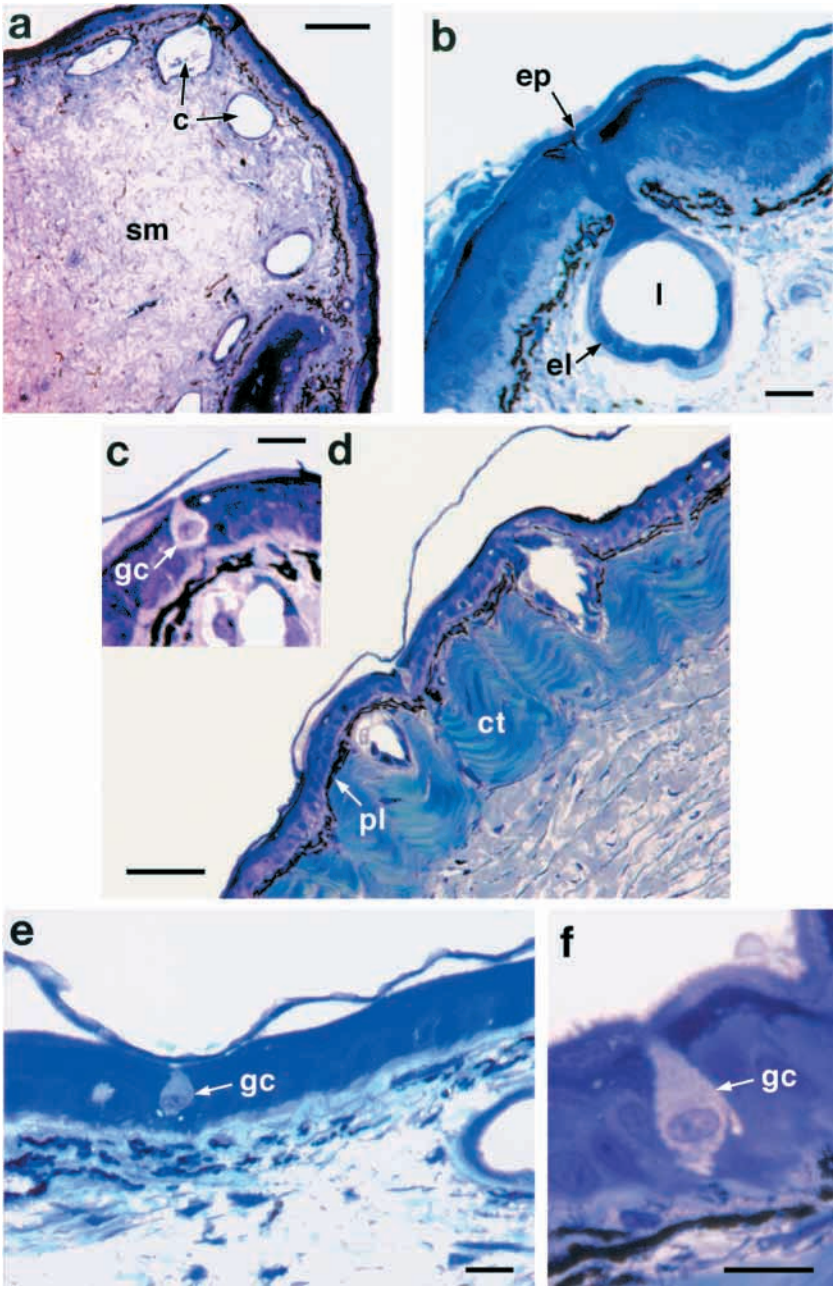

Fig. 5. Histological sections of the tympanic papilla of *Petropedetes parkeri* stained with Toluidine Blue and osmium. (a) Light micrograph of a longitudinal section through the tip of the papilla. Relatively large, peripherally located chambers or vesicles (c) surround the spongiform matrix (sm). (b) Chamber with epithelial lining (el) of the lumen (l). A connection from the chamber leading to the surface is apparent, presumably terminating in an exit pore (ep). (c) A goblet cell (gc) located at the papillar surface, with a pigmented layer (black) below the basal surface of the cell. (d) Low-magnification of the papillar surface showing chambers nearly surrounded by connective tissue (ct, wavy appearance) and the pigmented layer (pl). (e,f) Higher magnification of two goblet cells (gc) located in the outer surface layer of the papilla. Scale bars: a,d, 50 μm; b,c,e,f, 10 μm.

velocity of the tympanic papilla was always less (N=3) than that of a point diametrically opposite to the tympanic papilla on the ventral tympanic membrane, the velocity amplitude difference being greatest for frequencies between 1.0 and 2.0 kHz. For low-frequency stimulation (<0.4 kHz), the tympanic membrane is presumably vibrating in its lowest mode, and velocity differences between the tympanic papilla and the tympanic membrane tissue immediately surrounding the tympanic papilla will therefore be minimal.

Next, the relative velocity amplitude of a point at the center of the eardrum of a female *P. parkeri* was measured and its velocity compared with that measured after a small mass (10 mg; equal to approximately twice that of an average male tympanic papilla) was added to the eardrum of the female at the approximate location of the male tympanic papilla (Fig. 8). For the female on which this experiment was performed, adding mass to the tympanic membrane resulted in upward frequency shifts in the relative velocity peaks at the center of the tympanic membrane. It is of interest that, in a different animal, placing a small copper wire ring (mass 13.3 mg) over the tympanic papilla of the male resulted in no significant frequency shift in the first peak of its velocity response (at 680 Hz) but did cause an approximately 7.5 dB reduction in tympanic papilla velocity amplitude (data not shown).

In Fig. 9, the impulse response of the tympanic membrane of an intact male is compared with the mean (N=6) power spectral density function (PSDF) of the advertisement call of the male. Also in this figure, the velocity responses of a point

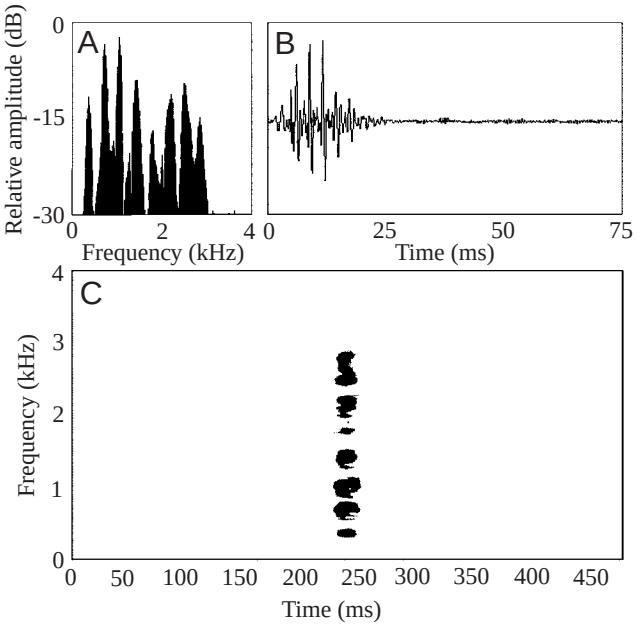

Fig. 6. Sonagraphic analysis of the advertisement call of *Petropedetes parkeri*. (A) Amplitude spectrum, (B) waveform of one call (amplitude *versus* time), (C) sound spectrogram. Both A and C illustrate the clear harmonic structure of the call, with the dominant frequency at approximately 1.0 kHz. In other individuals, the second harmonic is the dominant frequency, at approximately 700 Hz. The temperature during the recording was 22.5 °C.

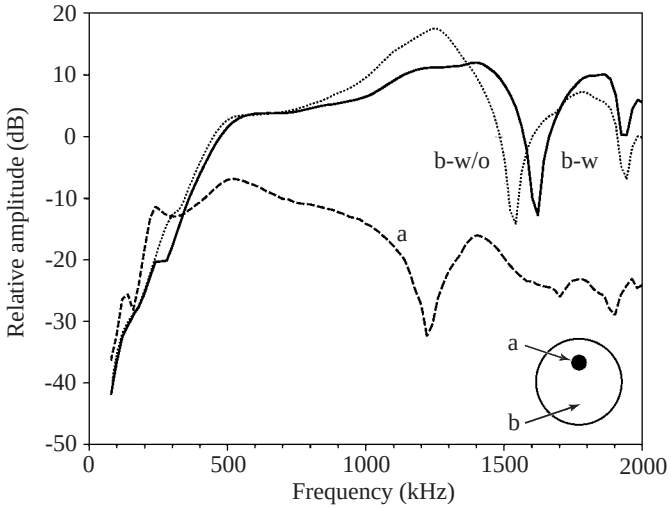

Fig. 7. Velocity amplitude at two points on the eardrum of a male *Petropedetes parkeri*, at the tip of the tympanic papilla (a) and at a point on the eardrum diametrically opposite the papilla location (b). Measurements were made under two experimental conditions: 'w', the intact eardrum with the papilla; 'w/o', the eardrum without the papilla (papilla surgically removed). The slight increment in the resonant frequency under load is attributed to the increase in tension generated by the deflection of the membrane. Vertical offsets represent actual differences in response.

at the center of the tympanic membrane of a male and female *P. parkeri* are shown for comparison. The frequency for which the power spectrum of the impulse response has its peak amplitude is 737 Hz, the dominant frequency of the average PSDF of the advertisement call of the male is 713 Hz and the frequency of the peak velocity of a point at the center of the tympanic membrane of a male is 700 Hz. These values fall quite close to one another, within a range of 37 Hz. In contrast, the frequency of the peak velocity of a point at the center of the tympanic membrane of a female is 800 Hz. Predictions of this, obtained using the simple approximation that the

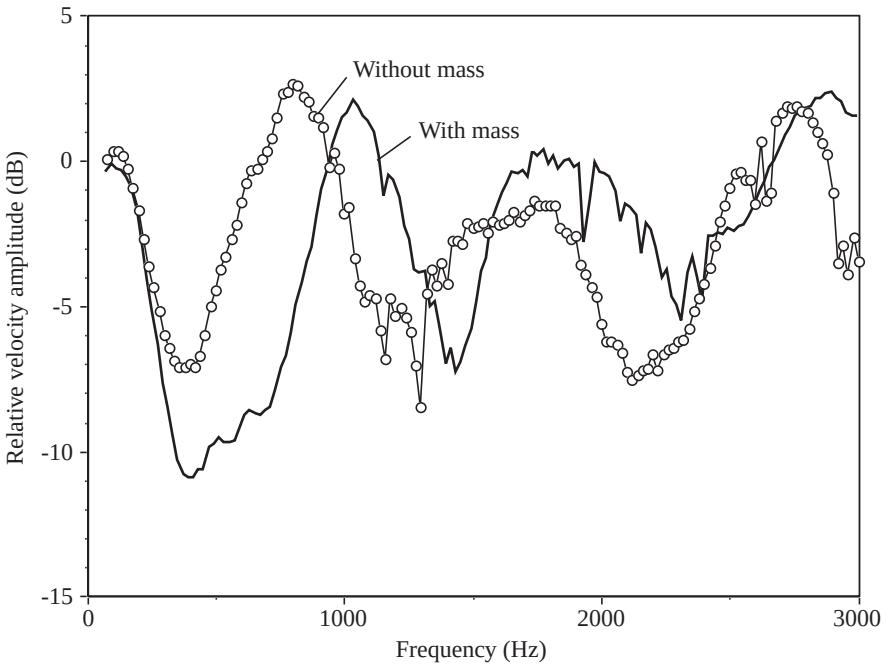

Fig. 8. Relative velocity amplitude of a point in the center of the eardrum of a female *Petropedetes parkeri* showing the effects of adding a small mass (10 mg) at the approximate location of the male papilla. Open circles, unloaded response; solid line, response after load placement. The increment in the peak frequencies is attributed to an increase in tension generated by the deflection of the membrane under load.

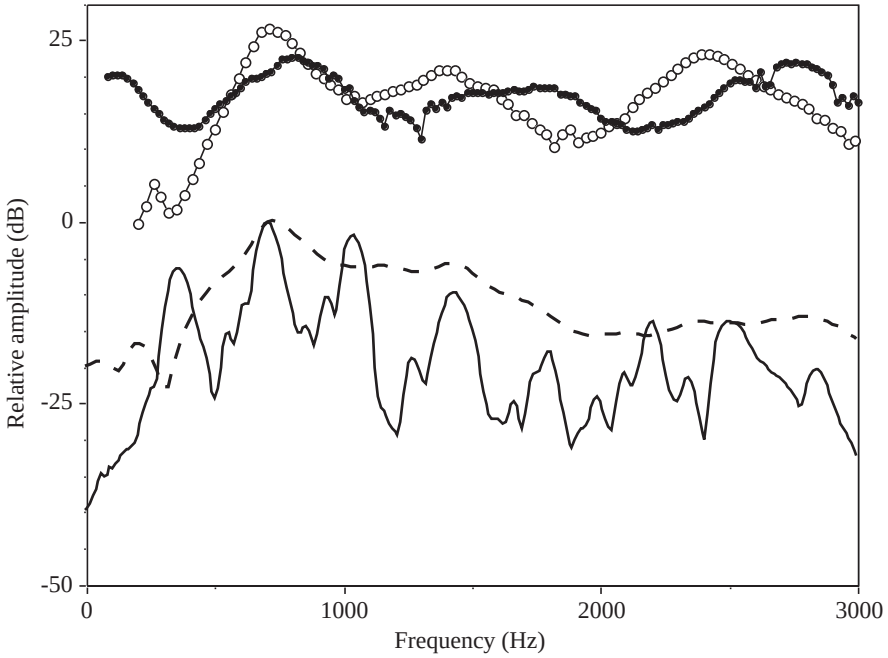

Fig. 9. A comparison between the average power spectrum (*N*=6) of the advertisement call (black curve, bottom), the Fourier Transform of the impulse response of the male tympanic membrane (dashed curve), the velocity response of a point in the center of the eardrum of a male (open circles) and the response of a point in the center of the eardrum of a female (filled circles). All units are in relative dB, and vertical offsets are arbitrary.

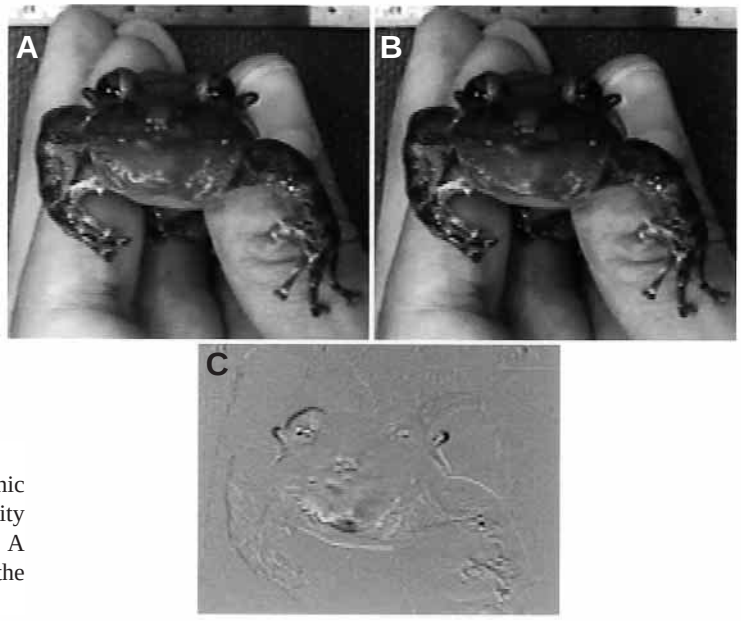

Fig. 10. Effects of buccal pumping on the position of the tympanic papilla during normal breathing (A) with the buccal cavity pressurized, and (B) with the buccal cavity depressurized. (C) A digital subtraction of the images in A and B emphasizing the structures that move during breathing (they appear darker).

fundamental frequency peak of the tympanic membrane scales inversely to its radius (the radius of the tympanic membrane of this female was 2.66 mm, whereas the radius of the tympanic membrane of the male was 3.37 mm), fall within 11 % of the measured value.

We also observed that, during normal positive-pressure breathing, the transient pressurization of the middle ear cavity causes the tympanic membranes to bulge outwards, resulting in obvious in-and-out tympanic papillar movements visible to the naked eye. By digitally subtracting two video images taken during normal buccal pumping, we were able to visualize the tympanic membrane movement (Fig. 10).

Discussion

The principal result of our morphological analysis is the confirmation that the tympanic papilla in this species is neither a columella nor the distal extension of such a structure. Rather, it is a fleshy protuberance that consists of a spongiform cellular matrix surrounded by a cuticular layer that is cellularly diverse, comprising at least four distinct cell types. Since the tympanic papilla adorns only the eardrums of the male during the breeding season, it is a clear example of an ephemeral secondary sexual characteristic.

Our histological results reveal the presence of a number of hollow, subsurface vesicles that resemble simple, multicellular

exocrine glands, alveolar in shape. A conspicuous and unusual component of courtship behavior in this species involves the female striking the male in the head region during amplexus with her foreleg (L. R. Minter, P. J. Bishop, P. M. Narins, E. R. Lewis and D. P. Lawson, in preparation). We speculate that this behavior is consistent with the hypothesis that the glands in the male tympanic papilla secrete a volatile substance that is released when the papilla is mechanically disturbed by the female during amplexus. This hypothetical substance might function as a pheromone to excite the female during mating behavior. It is of interest that, in this species, males but not females are known to possess femoral glands (Amiet, 1983), which are thought to release secretions that stimulate ovulation or ovipositional behavior by the female (Duellman and Trueb, 1986). Additional experiments are needed to test directly the hypothesis that the tympanic papilla is a source of pheromones.

In a recent study, Purgue (Purgue, 1997) found that the eardrums of the male North American bullfrog *Rana catesbeiana* radiate approximately 90 % of the energy in the release call of that species. Purgue predicted that call radiation by the tympanic membranes would probably be found both in other ranids and in species in which, like *R. catesbeiana*, the eardrums exhibit a sexual size dimorphism such that male eardrums are larger than those of the female. In *P. parkeri*, an African ranid, the area of the male eardrum is, on average, 126 % that of the eyeball, whereas the area of the female eardrum is 85 % of the area of the eyeball (Amiet, 1983). Thus, the two conditions predicted by Purgue (Purgue, 1997) to be associated with call radiation from the amphibian eardrum are satisfied for *P. parkeri*. Other potential radiating structures include the skin overlying the lungs (Narins et al., 1988) and the floor of the mouth cavity (males of this species do not possess an inflatable vocal sac).

The values we obtained for the peak vibration frequency of the intact male eardrum of *P. parkeri* measured using two separate techniques were within 5 % of each other. First, using laser Doppler velocimetry, we found that the maximum eardrum velocity amplitude in response to sweeping a pure tone injected into the mouth cavity was 700 Hz (Fig. 9). Next, we obtained the impulse response of the eardrum and determined that its Fourier transform exhibited a spectral peak at 737 Hz (Fig. 9). These two values correspond closely to the dominant energy in the advertisement call of the male (713 Hz, Fig. 9), consistent with the idea that the eardrum of *P. parkeri* serves as a transducer to receive (and/or broadcast) at least part of its advertisement call (Purgue, 1997).

We hypothesize that the observed upward shift in the peak vibration frequency of the female eardrum in response to off-center mass loading is the result of an increase in the tension in the eardrum, perhaps caused by membrane deflection. The compliant nature of the tympanic membrane means that adding a point mass may increase the tension of the membrane and locally increase its impedance, resulting in a decrease in the velocity amplitude at the point of application of the mass. This hypothesis is consistent with the measured vibrational behavior of the male tympanic papilla, where the large inertia presented by the papilla apparently accounts for the difference in velocity amplitude of this structure relative to the eardrum. In addition, although mass-loading the female eardrum raises its resonant frequency, whereas surgical removal of the tympanic papilla lowers the resonant frequency of the male eardrum, we can regard both these results as a manifestation of changes in membrane tension. In the female, the presence of the additional mass deflects the eardrum, causing a small amount of mechanical deformation that is necessarily accompanied by increased membrane tension, elevating the resonant frequency of the membrane. In the male, the tympanic papilla goes through the membrane, and it is largely supported by attachments to the periotic bone, thus causing little deformation or load on the intact eardrum. It is possible that surgical removal of the tympanic papilla resulted in a slight tearing of the underlying connective tissue, thus releasing tension on the membrane and, consequently, lowering its resonant frequency.

It is tempting, given the location of the papilla on the eardum, to ascribe a purely auditory function to the tympanic papilla. We have demonstrated that surgical extirpation of, or adding additional mass to, the papilla changes the vibratory properties of the eardrum. Nevertheless, during normal positive-pressure breathing, the buccal cavity pressure in the North American bullfrog (*Rana catesbeiana*) increases dramatically (Gans, 1974), resulting in an outward bulging of the tympanic membrane. We have observed the same phenomenon in the present study, but in *Petropedetes parkeri*, this bulging results in lateral motion of the tympanic papillae. Movement of the papillae during normal breathing is clearly visible in the laboratory to an observer (Fig. 10) and could serve as a cue in species recognition by females. Low ambient light levels do not necessarily preclude the use of visual signals (Hödl and Amézquita, 2001). It is known, for example, that despite the dogma that visual communication is inefficient at low light levels and at sites with high vegetation density (Harper, 1991; Endler, 1992; but for low-illumination prey detection in tree frogs, see Buchanan, 1998), visual signals are used by a variety of species over short distances in precisely these conditions, especially when combined with acoustic signals (McDiarmid and Adler, 1974; Richards and James, 1992; Hödl and Amézquita, 2001). We believe, however, that the dark coloration of the papillae and the fact that they move little during breathing both weigh against their use as a purely visual signal. Nevertheless, it remains to be tested whether the tympanic papilla is a secondary sexual characteristic that can facilitate the multimodal communication (olfactory, acoustic and visual) that underlies the reproductive and courtship behavior of *P. parkeri*.

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