Evolution of Calls and Auditory Tuning in the *Physalaemus pustulosus* Species Group

Walter Wilczynski[a]   A. Stanley Rand[b]   Michael J. Ryan[b, c]

Department of [a]Psychology and Institute for Neuroscience, University of Texas at Austin,
[b]Smithsonian Tropical Research Institute, Balboa, Republic of Panama, and [c]Section of Integrative Biology,
University of Texas, Austin, Tex., USA

Abstract

In species within the *Physalaemus pustulosus* species group, male frogs produce a whine-like advertisement call consisting of a frequency sweep typically descending from 1,000 to 400 Hz (depending on the species). One species, *Physalaemus pustulosus*, the túngara frog, has evolved a second call syllable, the chuck, which males place after their whine. Most energy in the chuck is above 1,500 Hz and peaks at 2,400 Hz. We investigated whether the evolution of this new call component in *P. pustulosus* coincided with evolution of auditory tuning. We used multiunit electrophysiological recordings of auditory-evoked activity in the midbrain to characterize auditory tuning in *Physalaemus pustulosus*, four other *Physalaemus* species within the *P. pustulosus* clade, and three additional, closely related *Physalaemus* species as outgroups. All eight species had similar sensitivity profiles, with a broad area of enhanced sensitivity from 100 to 1,100 Hz, which we presume represents amphibian papilla (AP) tuning, and a second, narrower area of enhanced sensitivity centered above 2,100 Hz, which we presume represents basilar papilla (BP) tuning. For all species, the whine stimulates the AP. The *P. pustulosus* chuck stimulates the BP. The frequency with greatest AP sensitivity differed significantly among species. Although in all cases the AP peak lay within the frequency sweep of the whine, phylogenetically corrected correlations revealed no significant relationships between AP tuning and any spectral feature of the whine. BP tuning was similar among all species, with mean BP best excitatory frequencies (BEFs) around 2,100–2,200 Hz, with the exception of *P. pustulatus*, with a mean BP BEF of 2,549 Hz. *Physalaemus pustulosus*, the only investigated species that produces a call component stimulating the BP, had a BP BEF that was not significantly different from any of the species within its clade except *P. pustulatus*, or from any of the outgroup species. A phylogenetic reconstruction of ancestral BP tuning confirms that the only point of evolutionary change in BP tuning is in the line of descent leading to *P. pustulatus*, not in the line leading to *P. pustulosus* despite this being the species using the BP for communication. The results indicate that BP tuning around 2,200 Hz is a conserved trait in the *Physalaemus pustulosus* species group and that no evolution of BP tuning accompanied the subsequent evolution of the call component (the chuck) that stimulates it. This supports the sensory exploitation idea, which posits that signals evolve to match preexisting features of receiver systems.

Copyright © 2002 S. Karger AG, Basel

Walter Wilczynski, PhD
Department of Psychology
330 Mezes Hall, University of Texas
Austin, TX 78712 (USA)
Tel. +1 512 475 8499, Fax +1 512 471 5935, E-Mail wilczynski@psy.utexas.edu

Introduction

Communication systems require a correspondence between signal and receiver to function effectively. Many neuroethological studies have approached animal communication systems by examining how characteristics of a species-typical signal relate to the properties of the sensory systems that process it [Hauser, 1996; Bradbury and Vehrencamp, 1998; Carew, 2000]. This has been a particularly fruitful avenue of research when applied to acoustic communication. Examining how the spectral frequency components of a vocal signal relate to the tuning of the auditory system has been a major emphasis in the study of the acoustic communication system of anuran amphibians (frogs and toads) since Capranica [1965, 1966, 1968] suggested a 'matched filter' hypothesis for call recognition in bullfrogs *(Rana catesbeiana)*. Capranica showed that the tuning of the auditory end-organs (specifically, the best excitatory frequency, BEF, or the area of greatest sensitivity within the end-organs range of hearing) matched the frequency or frequencies with the greatest relative energy in the species' advertisement call. It was then proposed that call recognition results from stimulation of the peak sensitivity regions in characteristic ways. Capranica proposed this idea based on studies in bullfrogs, which have an advertisement call with two spectral peaks, each corresponding to the tuning of one of the two auditory end organs typical of amphibians (the lower frequency amphibian papilla, AP, and the higher frequency basilar papilla, BP). Subsequent studies of many other species have revealed advertisement calls that stimulate only the AP, stimulate only the BP, or, as for bullfrogs, stimulate both. As a general rule, however, Capranica's idea that sensory filters match call frequency peaks holds across anuran species regardless of the frequency spectrum of the call [Gerhardt and Schwartz, 2001].

Although these extensive studies of anuran communication systems support the general rule that peak call frequencies of the species advertisement call roughly match peak auditory sensitivities, it is also true that some fundamental characteristics of the auditory system are stable, and are likely to represent phylogenetically conserved traits. The most important of these is the presence of both an AP and a BP in all anuran amphibians regardless of whether the call stimulates only the AP, only the BP, or both end organs [Wilczynski and Capranica, 1984; Wever, 1985; Lewis and Lombard, 1988; Zakon and Wilczynski, 1988; Lewis and Narins, 1999; Gerhardt and Schwartz, 2001]. In fact, both AP and BP are present in primitive frogs, many of which lack conspicuous calls, as well as in the two other amphibian orders, salamanders and caecilians, which generally lack acoustic communication [Wever, 1985].

Several of the physiological characteristics of the auditory organs are also consistent among anurans regardless of the communication signals [for reviews see Wilczynski and Capranica, 1984; Zakon and Wilczynski, 1988; Lewis and Narins, 1999; Smotherman and Narins, 2000]. The AP is the lower frequency end organ, with a range of VIIIth nerve afferents innervating it that are tuned between ca. 100 Hz and ca. 1,000–1,200 Hz in advanced anurans, with the high frequency cut-off lower in primitive anurans. There is variation in the size and frequency range of the AP. This, however, mainly reflects phylogenetic grades of organization rather than species-level adaptations to call characteristics, although AP variation itself might constrain the evolution of call characteristics [Lewis, 1984; Ryan, 1986; Lewis et al., 1992]. Furthermore, frequency responses of the AP fibers manifest several nonlinearities, including difference tone excitation and suppression of lower frequency cells by higher frequencies. This latter phenomenon results in the low frequency AP fibers (generally below ca. 400 Hz) being 'inhibitable' by high frequency stimulation whereas the higher frequency AP fibers are classified as 'non-inhibitable.' Although Capranica [1965] demonstrated how inhibitability could function in call recognition in bullfrogs, subsequent work revealed that suppression was present in all anurans investigated regardless of the spectral composition of the call, and seems to be the result of some inherent mechanical nonlinearity in inner ear mechanics common to amphibians and similar to that in the ears of many vertebrates [Capranica and Moffat, 1980; Wilczynski and Capranica, 1984; Zakon and Wilczynski, 1988].

The BP is invariably tuned to frequencies higher than the AP pass-band. Its tuning range is more narrow, a reflection of a smaller number of hair cells and a similar, largely mechanically-based tuning characterizing all the cells [Capranica and Moffat, 1983; Lewis and Narins, 1999]. Furthermore, BP tuning is more variable among species than AP tuning [Capranica and Moffat, 1983; Zakon and Wilczynski, 1988]. BP BEF is related to body size, in that smaller frogs have, on average, BPs tuned to higher frequencies than larger frogs [Loftus-Hills, 1973; Shofner and Feng, 1981; Wilczynski et al., 1984; Zakon and Wilczynski, 1988; Keddy-Hector et al., 1992]. This often results in two distinct areas of enhanced frequency sensitivity in the anuran auditory system: one broad area below 1,000 Hz, often with low and mid frequency minima, reflecting the diverse hair cell populations of the AP,
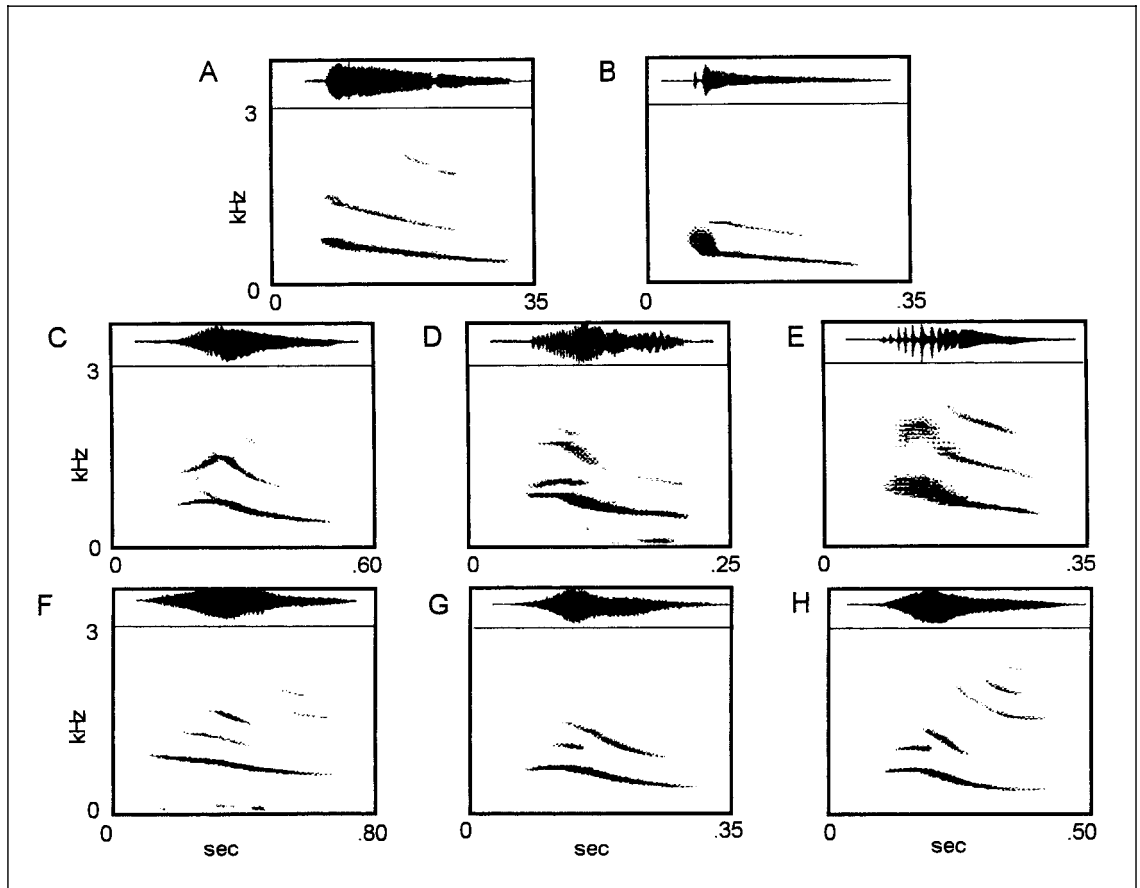
Fig. 1. Calls of *Physalaemus* species studied. Oscillograms are in top panel and sonograms in bottom panel for each species. **A** *P. pustulosus*, **B** *P. petersi*, **C** Species B, **D** *P. coloradorum*, **E** *P. pustulatus*, **F** *P. enesefae*, **G** *P. ephippifer*, **H** Species A.

and a more narrow, often higher threshold, area centered at higher frequencies that can range across species from less than 1,500 Hz in large anurans such as bullfrogs or large toads, to above 3,000 Hz in small hylids [Zakon and Wilczynski, 1988; Gerhardt and Schwartz, 2001].

The observation that many features of the anuran auditory system are phylogenetically conserved while often seeming to have species-specific characteristics that match aspects of conspecific calls raises questions about how the characteristics of signals and receivers evolve in communication systems. Such a relationship might result from coevolutionary processes, in which both signal and receiver change together [Alexander, 1962; Hoy et al., 1977; Doherty and Hoy, 1985; Boake, 1991], receivers could change to match communication signals once they are adopted [Borgia and Coleman, 2000], or signals may evolve to match preexisting receiver characteristics

[Ryan, 1990, 1998; Endler, 1992; Shaw, 1995; Endler and Basolo, 1998]. Furthermore, these processes can be sequential or interact within a lineage [Ryan, 1997]. The sensory exploitation hypothesis [Ryan, 1990] postulates the last scenario for the evolution of mate attraction signals, proposing that male advertisement signals evolve to match pre-existing biases in female sensory systems. Sensory exploitation is a subset of Endler's [1992] sensory drive idea, which suggests that the evolution of a signal is constrained by the receiver's sensory system and the biotic and abiotic environment in which it is transmitted. In the present study, we investigated the relationship between the spectral characteristics of advertisement calls and auditory tuning in members of the *Physalaemus pustulosus* species group to test these ideas about the direction of signal-receiver evolutionary patterns.

The anuran leptodactylid genus *Physalaemus* consists of a large number of species distributed widely throughout Middle and South America. One clade within the genus forms the *Physalaemus pustulosus* species group, named for its most well-studied species. *P. pustulosus* has been the subject of a long-term investigation of acoustic communication, mate choice, and sexual selection [e.g. Ryan, 1980, 1985; Ryan and Rand, 1998, 2001; Wilczynski et al., 1995, 1999]. All frogs in the *P. pustulosus* species group produce whine-like advertisement calls [fig. 1; Ryan and Rand, 1993]. These calls consist of frequency sweeps, moving from higher to lower frequencies. The species differ in the spectral and temporal parameters of their whine (fig. 1; table 1), but for all species the fundamental of the whine (in which the great majority of the call energy is located) overlaps only the frequency sensitivity of the typical anuran AP (i.e., frequencies are generally below 1,250 Hz). The whine stimulates female phonotaxis and is used in male-male vocal interactions [Ryan, 1985]. The whine's fundamental is both necessary and sufficient to elicit phonotaxis with the upper harmonics having no influence on phonotaxis. In the *Physalaemus* species studied, the whine is necessary and sufficient for both female and male responses.

Individual *Physalaemus pustulosus* males can add an additional component to the end of their whine, the 'chuck' (fig. 2). Up to six chucks may be added, and are done so usually in response to other male vocalizations. Females prefer calls with chucks. They also prefer lower frequency chucks produced by larger males and gain a reproductive benefit from doing so [Ryan, 1985]. The dominant frequency of the chuck averages 2,400 Hz, and 90% of its energy is above 1,500 Hz. As expected from the general pattern across anurans, and as confirmed by behavioral and neurophysiological studies, the chuck primarily stimulates the BP rather than the AP, and the precise relationship between tuning and call frequency is consistent with female preference for lower frequency chucks [Ryan et al., 1990; Wilczynski et al., 1995; Ryan and Rand, 2001].

P. petersi is the sister species of *P. pustulosus* (fig. 3), although *P. petersi* might actually represent a species complex [Cannatella et al., 1998]. Some populations of this taxon add call suffixes whereas others do not. None of the other species in the species group, nor any of the more than 20 other species we have surveyed in the genus, add suffixes. Thus *Physalaemus pustulosus* uses both the AP and the BP for conspecific communication, while most other species only use the AP. The distribution of call characters within the clade implies that the lack of a chuck (and hence the lack of BP involvement in communication) is the ancestral condition within this species group, and the use of a BP-stimulating suffix evolved in *Physalaemus pustulosus* or the common ancestor of *P. pustulosus* and *P. petersi* [Ryan et al., 1990; Ryan and Rand, 1993]. Ryan et al. [1990] showed that BP tuning in *P. pustulosus* and another species in the species group, *P. coloradorum*, were not significantly different from one anoth-

Table 1. Mean values of spectral call characters, most sensitive frequencies of the amphibian (high peak) and basilar papillae, and snout-vent length of frogs of the *Physalaemus pustulosus* species group and closely related species. Call parameters were assessed for the whine component of the call only, not the obligatory amplitude modulated prefix of *P. pustulatus* or the facultative 'chuck' suffix of *P. pustulosus*

Species	MXHZ	FNHZ	TMHFHZ	DOMHZ	BPBEF	APHI	SVL
enesefae	976	692	0.518	962.4	2,157.0	632.8	26.3
ephippifer	944	576	0.526	994.0	2,178.0	688.5	24.4
Species A	876	460	0.473	983.4	2,101.1	650.6	29.2
pustulosus	884	484	0.335	712.0	2,133.3	516.7	26.8
petersi	1,220	384	0.116	627.9	2,166.0	629.0	22.9
Species B	888	444	0.292	894.3	2,218.0	730.5	26.3
coloradorum	1,180	628	0.387	1,113.2	2,228.6	528.5	21.9
pustulatus	964	676	0.429	1,062.0	2,549.2	717.0	23.7

Abbreviations and units of measurement: MXHZ, maximum frequency (Hz); FNHZ, final frequency (Hz); TMHFHZ, time to mid-frequency (ms); DOMHZ, dominant frequency (Hz); BEF, most sensitive frequency of the basilar papilla (Hz); APHI, most sensitive frequency of the high peak of the amphibian papilla (Hz); SVL, the mean snout-vent length of the frogs used in the neurophysiological study (mm).

Fig. 2. An oscillogram (**A**) and a sonogram (**B**) of a whine plus three chucks of the túngara frog, *Physalaemus pustulosus.* Power spectra of the whine (**C**) and chucks (**D**) are shown below. The relative amplitudes of frequencies in **C** and **D** are on a linear scale, although the magnitude of **C** is much greater than **D**. The dominant frequency peak in **D** results from the fundamental frequency of the whine, which passes through the second harmonics of the three chucks.
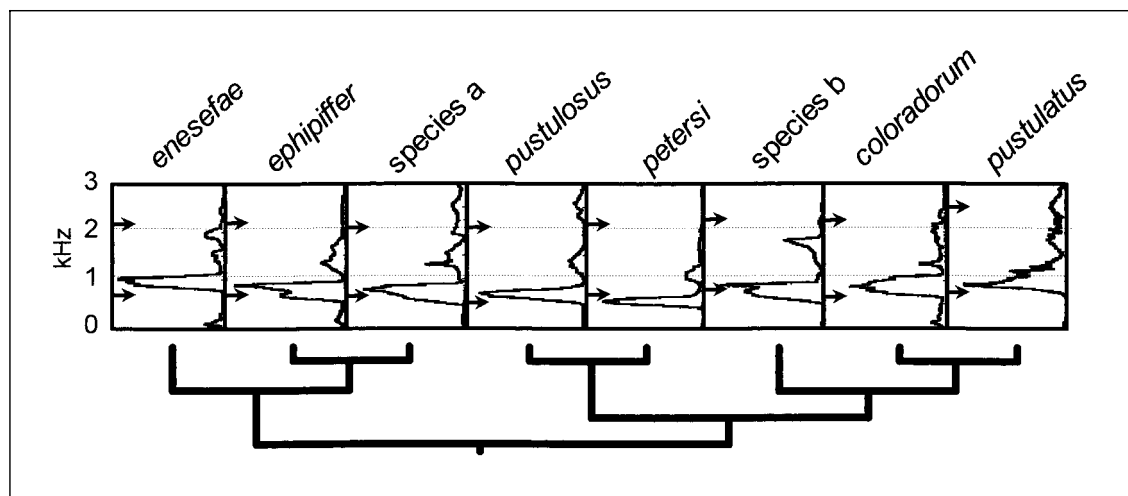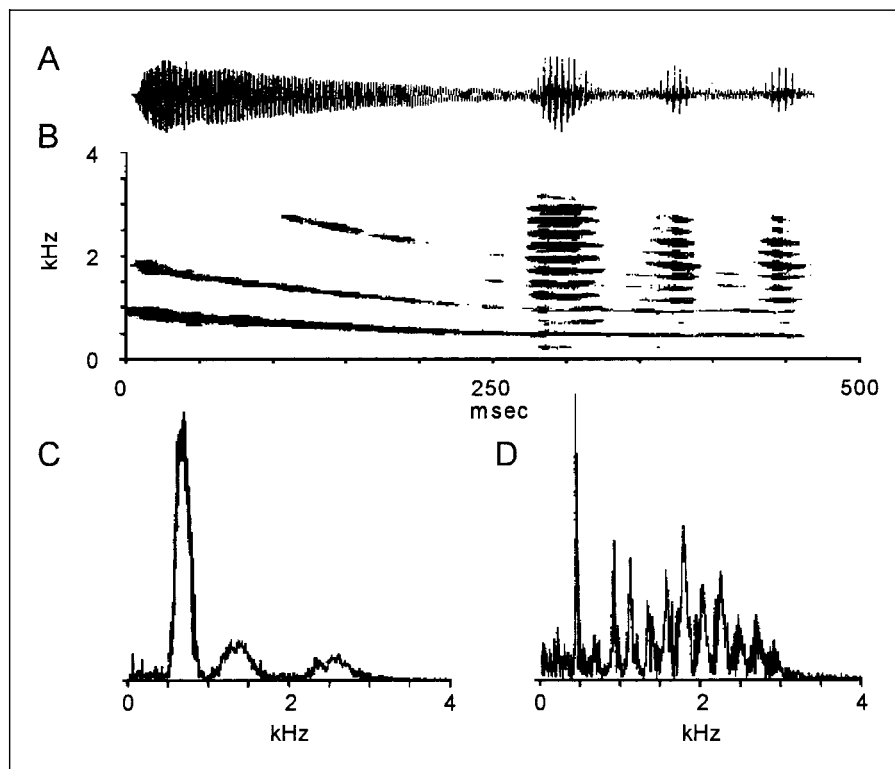

Fig. 3. Fourier transforms of typical calls of the *Physalaemus pustulosus* species group and the three congenerics used for outgroups. The relative amplitudes of frequencies are on a linear scale. For *P. pustulosus* a whine only with no chucks (cf. fig. 2) is analyzed, as in table 1. An entire call of *P. pustulatus,* including the obligatory amplitude modulated prefix, is analyzed here, whereas only the whine component of the call is analyzed in table 1. Arrows show the mean best excitatory frequency of the amphibian and basilar papillae for each species. The phylogenetic relationships among species are illustrated by the branching diagram.

er. They argued, therefore, that the BP tuning in *P. pustulosus* did not evolve to match the spectral characteristics of the chuck or coevolve with the chuck, but that the chuck evolved to exploit the preexisting tuning characteristics of the BP. Thus the benefit derived from female preference for large males, which results from their preference for lower frequency chucks [Ryan 1985], might be implicated in the evolutionary maintenance of BP tuning in *P. pustulosus*, but not in its origin. Ryan and Rand [1993] also showed that *P. coloradorum* females prefer conspecific calls to which chucks of *P. pustulosus* have been appended to the normal conspecific call, further supporting the hypothesis of sensory exploitation.

The distribution of call characters within the *P. pustulosus* species group provides us with the opportunity for a phylogenetically controlled investigation into the relationship of signal characteristics and auditory tuning in order to explore further any evidence for shifts in receiver tuning as a consequence of signal evolution. To do this, we characterized midbrain auditory tuning in eight species, five within the *P. pustulosus* species group and three other congeneric species used for outgroup comparison (fig. 3). We compared AP and BP tuning characteristics of species with their body size and spectral call characteristics. If BP tuning had evolved coincident with the introduction of a new signal character, we predict BP tuning in *Physalaemus pustulosus* to be different from BP tuning in the other species in the clade. Similar tuning in all species in this species group would indicate a phylogenetically conserved pattern of receiver characteristics to which the specialized call suffix of *P. pustulosus* evolved to match.

Materials and Methods

Neurophysiology of the Auditory System

Auditory system tuning, as assessed by multiunit recordings in the midbrain (see below) was characterized in eight species, five within the *Physalaemus pustulosus* species group, *P. pustulosus* (n = 6), *P. petersi* (n = 4), *P. coloradorum* (n = 7), Species B (n = 9; species not formally described but see Ryan and Rand [1995]), *P. pustulatus* (n = 8), and three congenerics used as outgroups, *P. ephippifer* (n = 2), Species A (n = 10; species not formally described), and *P. enesefae* (n = 9). Two *P. pustulosus* and five *P. coloradorum* were females; all others were male. All individuals were collected at sites in Middle and South America (*P. pustulosus*, Panama; *P. enesefae*, Venezuela; Species A and *P. ephippifer*, Brazil; *P. coloradorum, P. pustulatus* and *P. petersi*, Ecuador; Species B, Peru) during the course of field studies investigating the communication systems, systematics, and biogeography of *Physalaemus* [Ryan and Rand, 1993, 1995, 1998, 1999, 2001; Cannatella et al., 1998].

Each species represented in this study included animals collected from only one site to avoid potential intraspecific geographic varia-

tion. Differences in the number of subjects and sex ratio in each species sample were due to a variety of reasons including permit restrictions, difficulty in collecting, differences in successfully transporting individuals from distant sites, and failure to meet criteria for data inclusion following the neurophysiology experiments (see below). Individuals were held for varying periods of time post-collection, depending on the constraints of the field studies, before being hand-carried back to the University of Texas at Austin for neurophysiological studies. Upon arrival, frogs were held in the laboratory for a minimum of two weeks to allow recovery from the stress of transportation. During that time, they were held in aquaria, under a 12:12 light/dark cycle, and fed insects (fruit flies or small crickets) ad libitum.

For the neurophysiological characterization of auditory tuning, a frog was anesthetized by immersion in a 2.5% aqueous solution of urethane. Skin on the top of the head was carefully incised and retracted, and a hole drilled in the skull overlying the midbrain. The skin was repositioned and the animal allowed to recover from the anesthesia and surgery for 2 days with careful monitoring for signs of any adverse reactions or compromised condition. Following this recovery, the animal was injected with d-tubocurarine chloride (intramuscular, 7.5 µg/g body weight in 20 mg/ml aqueous solution) and treated with 2% lidocaine as a local anesthetic. The animal was placed on a cork platform atop a vibration-reducing table inside an Industrial Acoustics sound attenuating booth. An earphone assembly was sealed around one ear. The earphone was calibrated after the experiment with a B&K 2230 precision digital sound level meter. A drawn glass microelectrode, filled with 3 M KCl, with tip broken to yield an impedance of 3–5 MΩ, was placed in the midbrain contralateral to the earphone. The electrode was lowered with a hydraulic microdrive until robust multi-unit auditory activity could be discerned in response to a multi-tone search stimulus. Once such activity was isolated, single frequency tone bursts (150 ms duration, repeated every 1.5 s) were presented. Tone frequencies ranged from 100 Hz to 4,000 Hz, in 100- or 200-Hz intervals.

Thresholds to each tested tonal frequency were determined by monitoring the neural activity through earphones and on a storage oscilloscope. Signal amplitude was adjusted up and down in 10-dB, then 1-dB increments using manually controlled resistive attenuators until acoustically driven multiunit activity could no longer be discerned. The resultant frequency-threshold curve was ultimately used to construct audiograms for each recording position, following correction using the calibration curve for the sound delivery system. Once approximate threshold minima for the AP and BP were determined by this method, tuning was more accurately determined using smaller frequency steps until an estimate of the BP BEF and the location of peaks of AP sensitivity corresponding to the 'low' and 'high' frequency AP populations were obtained. A minimum of two audiograms was obtained for each electrode position. A minimum of two electrode positions with clear auditory activity was required for a frog to be included in the data analysis (most frogs had recordings from 4 or more positions within the midbrain; there was no evidence of tonotopy in our recordings). Frogs whose recordings did not meet these criteria were excluded from the analysis, resulting in the subject number for each species reported above. Not all individuals yielded clear BEFs for both AP and BP. For each individual frog, the data were averaged to obtain individual mean BP BEF and AP sensitivity peaks. Individual means were then averaged to obtain species means.

Following the recording session, the snout-vent length of the frog was measured with dial vernier calipers.

Call Characters

Male advertisement calls for each species were recorded at the same field sites from which the animals used in the neurophysiology experiments were collected (although not necessarily from the same individuals). Calls were recorded with either Marantz model PMD 420, Sony model TCD 5M, or Sony Professional Walkman tape recorders and Sennheiser ME 80 microphones with K3U power modules. Calls were usually recorded at ca. 50 cm from the calling male. Calls were digitized at a sampling rate of >20 kHz and analyzed with the software program Signal [Beeman, 1996]. Data presented here on the call's spectral frequency were determined by fast Fourier analysis and spectral contour analysis of the whine's dominant frequency [see Beeman, 1996]. A more complete description of the call variation within and among the species can be found in Ryan and Rand [1993].

The advertisement calls of all eight species studied here are frequency sweeps, or 'whines' (fig. 1). From these studies, we calculated mean values of four spectral call characters: the maximum frequency of the whine; the final frequency of the whine; the dominant frequency of the whine; and an estimate of the shape or time constant of the whine, the proportion of the whine's duration to the mid-frequency of the whine. We also calculated the mean dominant frequency of the *P. pustulosus* chuck (fig. 2) for this species.

Statistical Analysis

We restricted our statistical analysis to the BP BEF and the high frequency AP sensitivity peak, as the low frequency AP sensitivity peak was less clearly observable in all individuals and was lower than the low frequency end of the whine in all species.

We compared the BP and AP tuning among all species, and between *P. pustulosus* versus all other species with a one-way analysis of variance. We included snout-vent length as a covariate because BP tuning was correlated with body size. We used a Tukey HSD multi-comparison test for post hoc analysis. Because we are interested in the lack of differences in tuning between *P. pustulosus* and other species, we performed power analyses when analyses of variance failed to reveal significant differences. We also conducted Pearson product moment correlations among pairs of variables. Statistical analyses were conducted using Systat 9.0 [Wilkinson, 1996].

We used the software Compare version 4.3 [Martins, 2001] to compute phylogenetically independent contrasts [Felsenstein, 1985] to test hypotheses of correlations between species' size, auditory tuning, and call characters. This is necessary because species' characters are not evolutionarily independent data [Felsenstein, 1985]. A species' phenotype results from a combination of traits shared by other species due to inheritance from a common ancestor and traits more recently evolved in that particular species. Therefore, when comparing traits, or the correlation of traits, among species one is comparing data that are partly independent and specific to each species and partly shared among related species due to common inheritance [Martins and Hansen, 1997]. Independent contrast methods estimate the shared component of the data from known phylogenies and measured values in extant species, then use this estimate to transform the data into a normally distributed set of parameters called 'independent contrasts' that represent the variation among species after the effect of phylogeny is removed. Statistical comparisons among species, or correlations between variables among species (as calculated in our study), are then performed on the transformed data, thus satisfying the requirements of parametric statistics that groups must represent independent data points.
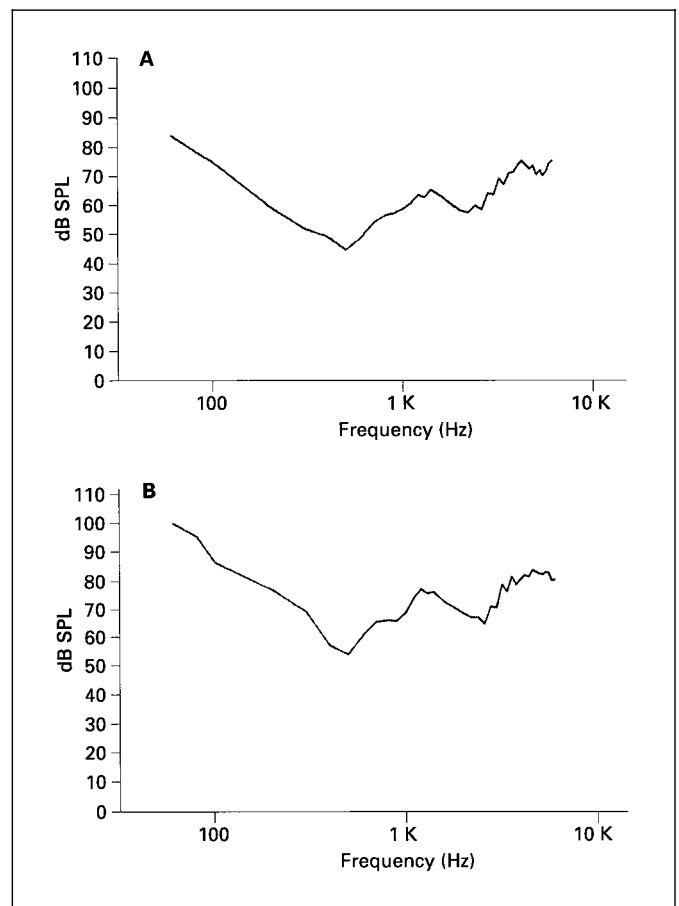

Fig. 4. Representative audiograms from *Physalaemus pustulosus* (**A**) and *P. coloradorum* (**B**). Each audiogram represents the threshold as a function of frequency for multiunit activity at a single midbrain recording location. In each, the lower frequency threshold minimum was considered the BEF of the mid-frequency AP fiber population and the upper frequency threshold minimum was considered the BEF of the BP.

We estimated BP and AP tuning for ancestral nodes of the phylogeny in order to illustrate possible patterns by which tuning evolved. These estimates were based on the generalized least squares approach described in Martins and Hansen [1997] and implemented in Compare 4.3 [Martins, 2001].

All procedures were approved by the University of Texas Institutional Animal Care and Use Committee.

Results

Midbrain-derived auditory tuning from all eight species had the typical appearance of anuran audiograms. Representative audiograms are shown in figure 4. A broad area of low and mid frequency sensitivity extending

Table 2. Phylogenetic correlations between tuning of the two auditory end organs and spectral call characters and body size for frogs of the *Physalaemus pustulosus* species group and closely related species. (All abbreviations are the same as in table 1.)

	APHI	BP
INHZ	−0.340	0.400
MXHZ	−0.230	−0.065
FNHZ	−0.076	0.571
TMHFHZ	0.069	0.363
DOMHZ	−0.170	0.211
SVL	−0.086	−0.330

APHi vs. BPBEF 0.648.

from 100–200 Hz to about 1,000–1,100 Hz was followed by an area of elevated thresholds leading to a secondary, higher frequency sensitivity that in all but one of these *Physalaemus* species was centered around 2,100–2,200 Hz. Within the broader lower band, there were two threshold minima in at least some members of each species. The lower of these, centered around 300–400 Hz, was more variable and less easily discerned in all individuals. The second was more consistent and pronounced in our recordings and was centered around 500–700 Hz. We presume that the two broad sensitivity regions reflect the tuning of the AP and BP, with the two minima in the lower AP band indicating the low frequency 'inhibitable' and mid frequency 'noninhibitable' AP populations described in other anurans. Because the low frequency AP minimum was beyond the range of call frequencies in all *Physalaemus* species included here, we did not include it in any of our statistical analyses, and will not discuss it further. BP and AP tuning were not significantly correlated (r = 0.228, n = 49, p = 0.110 for all individuals; r = 0.274, n = 8, p = 0.441 for species' means). The mean center frequency of the upper AP threshold minimum and the mean BP BEF, as well as the four mean call characters describing the whine and the mean snout-vent lengths of the subjects used in the study are shown in table 1.

Because species are not evolutionarily independent samples we conducted correlation analyses of the relationship between calls and tuning on phylogenetically independent contrasts. None of the correlations were statistically significant (all p > 0.05; table 2). The only suggestion of a possible correlation between tuning and call characters was BP tuning and the final frequency of the call. This is probably spurious, as the final frequency is the frequency most different from the tuning of the BP. The AP correlation with whine dominant frequency, which one might expect to match AP tuning, was very weak and in the wrong direction from the expected (r = −0.170) and thus does not indicate a tight match between call and tuning. Spectral call characters of the whine generally yielded moderate positive correlations with BP tuning higher than those found for the upper AP peak, with the exception of the maximum frequency of the whine, which has virtually no relationship to BP BEF (r = −0.065). There is a stronger correlation between BP and AP tuning in the phylogenetically corrected correlation (r = 0.648) than in the uncorrected correlations reported above.

Across all individuals snout-vent length was negatively correlated with the BEF of the BP (r = −0.430, n = 50, p = 0.002) but not with the upper AP sensitivity peak (r = 0.185, n = 48, p = 0.208). Results were similar when species means were analyzed, but the correlation between snout-vent length and BP BEF was not statistically significant given the small number of species (snout-vent length versus BP, r = −0.450, n = 8, p = 0.264; snout-vent length versus AP, r = 0.116, n = 8, p = 0.785; BP versus AP, r = 0.274, n = 8, p = 0.441). The phylogenetically corrected correlation between size and BP tuning was of a similar magnitude compared to the uncorrected, simple correlations (phylogenetic correlation r = −0.330). Because of the correlations between snout-vent length and BEFs, comparisons of tuning among species were made with an analysis of covariance to correct for the potential contribution of body size to species differences in tuning.

An analysis of covariance, with snout-vent length as a covariate, showed that the tuning of the upper sensitivity peak of the AP differed among species ($F_{7, 39}$ = 8.950, p < 0.001); there was no significant effect of the covariate ($F_{1, 39}$ = 0.016, p = 0.901), which is expected given the lack of significant correlations between body size and AP tuning shown above. Similar results were obtained when no covariate was used in the analysis ($F_{7, 39}$ = 10.686, p < 0.001). *P. pustulosus* had the lowest BEF of the AP. A Tukey HSD multiple comparison test showed that the 9 significant pairwise differences out of a possible 56 comparisons were distributed across all taxa. *P. pustulosus* differed from Species B (p < 0.001), *P. enesefae* (p = 0.013), *P. ephippifer* (p = 0.016) and *P. pustulatus* (p < 0.001). Species B differed (p < 0.05) from three species, *P. coloradorum* from two species, *P. petersi* did not differ from any species, and the others differed from one species.

An analysis of covariance, with snout-vent length as the covariate, showed that the tuning of the BP also dif-
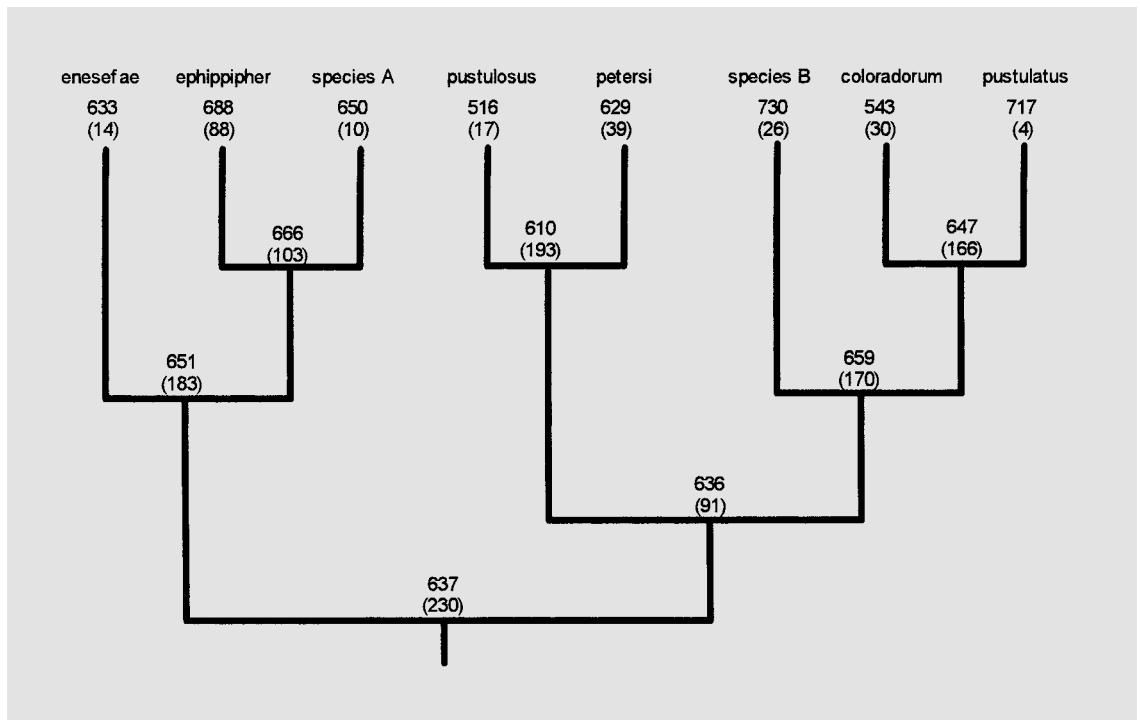
Fig. 5. Estimates of ancestral characters for AP tuning. There are no lineages with substantially greater evolutionary change, as defined by the difference between an ancestor and its descendent being greater than the standard error of the descendent.

fered among species ($F_{7,41} = 3.68$, p = 0.004); there was a significant effect of the covariate ($F_{7,39} = 7.405$, p = 0.010) as is expected from the univariate correlation analysis presented above. Similar results were obtained when there was no covariate ($F_{7,39} = 4.204$, p = 0.001). The posthoc analysis, however, reveals a very different pattern of pairwise differences among species than was evident in AP tuning. There were 6 significant differences (Tukey HSD multiple comparison test, p < 0.05) out of a possible 56 comparisons, and in 5 of these *P. pustulatus* differed from other species: Species B (p = 0.016), *P. coloradorum* (p = 0.025), *P. enesefae* (p = 0.002), *P. petersi* (p = 0.014) and *P. pustulosus* (p = 0.020). The other significant difference was between Species B and Species A (p < 0.001).

Because we are concerned with the hypothesis that there was no coevolution of BP tuning that accompanied the evolution of the chuck, more specifically that the tuning of *P. pustulosus* does not differ from the other species, we repeated the above analysis excluding the data from *P. pustulatus*, the species that was responsible for most of the differences in BP tuning in the previous analysis. The analysis of variance did not reveal statistically significant

differences in BP tuning between *P. pustulosus* and the pooled data from the other species ($F_{1,45} = 0.348$, p = 0.558). Small differences in mean BP tuning resulted in low power of the test to identify group differences (power = 0.067). Given the effect sizes of the populations (the difference between the means divided by the combined standard deviation), sample sizes of 243 for all other species and 264 for *P. pustulosus* would have been required to achieve a power of 0.80. Thus it is unlikely that the lack of significant differences between *P. pustulosus* and its close relatives results from small sample sizes.

We estimated the ancestral states of AP tuning, and asked where on the phylogenetic tree do major evolutionary changes in tuning occur (fig. 5). Our criterion for a major change is when the difference in tuning between an ancestor and its descendant is greater than the standard error of the estimated tuning of the ancestor. Given the large differences in AP tuning among many pairs of species, the standard errors of the ancestral estimates are quite large. Thus we cannot isolate particular lineages that exhibited more evolutionary changes in AP tuning than others.
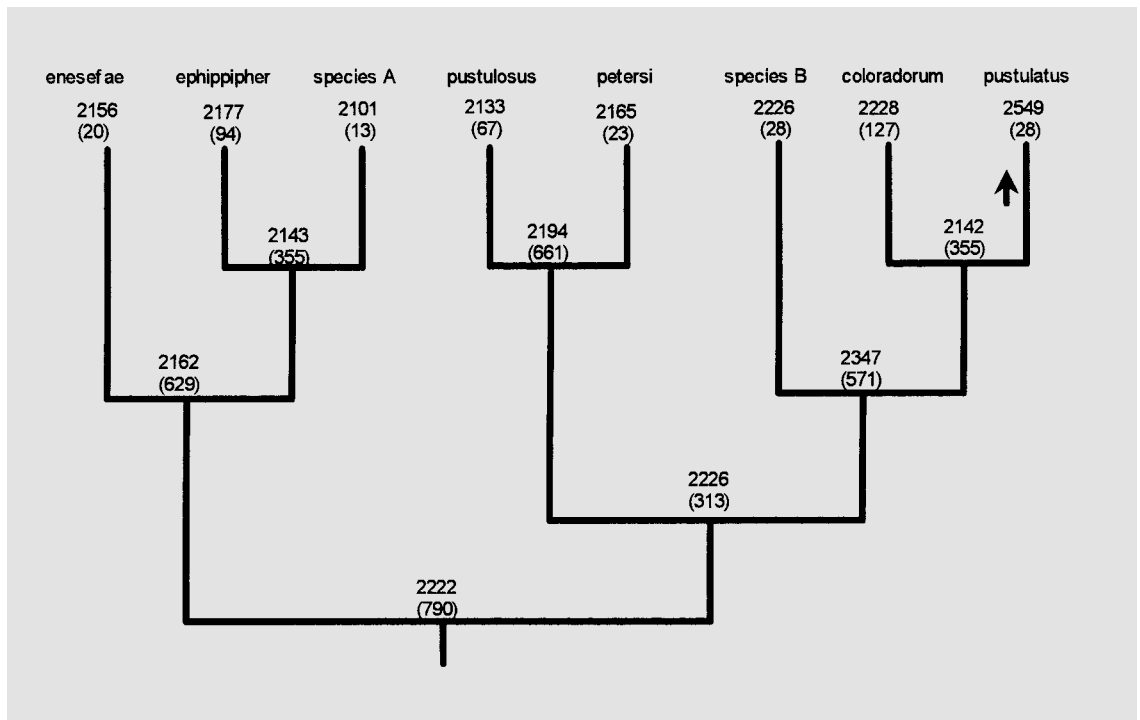
Fig. 6. Estimates of ancestral characters for BP tuning. The arrow shows the location of major evolutionary change, as defined by the difference between an ancestor and its descendent being greater than the standard error of the descendent.

We estimated the ancestral states of BP tuning (fig. 6). Using the criterion of a major evolutionary change as defined above, the only such change was along the line of descent from the immediate ancestor of *P. coloradorum* and *P. pustulatus* to *P. pustulatus*. This is consistent with the analysis of variance result that most statistical differences in BP tuning result from *P. pustulatus* differing from other species. Furthermore, there is little evolutionary change from the root of the phylogeny to the tips of all the other species, including *P. pustulosus*. Thus both the analyses of variance and the phylogenetic reconstruction of ancestral traits suggest that BP tuning is a relatively conserved characteristic, with no discernable evolutionary change in the line of descent leading to *P. pustulosus*.

Discussion

Within the *Physalaemus pustulosus* species group most species restrict their acoustic communication system to the frequency band of the AP. *P. pustulosus* is unusual in that it produces an AP stimulating frequency sweep (the whine) typical of the species group (fig. 1) but facultatively adds to it a higher frequency suffix, the 'chuck' (fig. 2), that stimulates the BP. The presence of the BP is an ancestral character present in all *Physalaemus* (and, more generally, all anurans and most amphibians). Thus the chuck is a derived trait that must have evolved after the emergence of the BP. Furthermore, the results show that the tuning of the BP, with a BEF around 2,200 Hz, is similar in all of the *Physalaemus* species examined with the exception of *P. pustulatus* (fig. 3, table 1). Phylogenetic reconstruction of ancestral nodes suggests those tuning differences evolved in the lineage between *P. pustulatus* and its immediate ancestor (fig. 6).

Given the tuning similarity across all species, the most parsimonious assumption is that BP tuning to this frequency band is also the ancestral trait of the *Physalaemus pustulosus* species group, and therefore the *P. pustulosus* chuck evolved after this particular BP tuning arose. Nevertheless, the emphasized frequencies in the chuck approximately match BP tuning. If the phylogenetic assumptions are correct, the general congruence between BP tuning and the emphasized frequencies in the chuck

seen in *P. pustulosus* is not a result of a coevolutionary process of signal and receiver change, nor the result of a shift in receiver characteristics towards signal characteristics, but seems to result from the spectral properties of the chuck evolving to exploit or match the tuning of a preexisting auditory organ.

Our results, in fact, indicate that there has been little evolution of basic auditory tuning properties related to call spectral properties in this anuran clade. This is most obvious for the BP. Its tuning does not differ within the species group, except for *P. pustulatus*, despite the fact that only one of the investigated species, *P. pustulosus*, uses it for intraspecific communication. (Some populations of *P. petersi*, broadly defined, add call suffixes, but these populations were not investigated.) There are some statistically nonsignificant but moderate positive correlations, but not matches, between BP tuning and some parameters of the whine (table 2). These are likely spurious, or at least of uncertain relation to any communication function, as in no case do any of the whine frequencies approach the sensitivity band of the BP. Moreover, the strongest correlation is between BP tuning and final whine frequency, which is the lowest whine frequency and therefore furthest from the BP's sensitivity.

AP tuning differs among *Physalaemus* species and these differences occur between many pairs of species, unlike the differences in BP tuning, which are mostly confined to the difference between *P. pustulatus* and some other species. There is little systematic relationship between AP tuning and whine spectral measures, other than the fact that in all cases the whine sweeps through the upper portion of the AP, and in nearly all species through the upper frequency sensitivity peak. Correlations between call spectral parameters and the upper threshold minimum of the AP, however, are all statistically nonsignificant and weak. Furthermore, all are negative rather than positive as one would predict if calls and tuning were matched. It seems unlikely that the lack of a match is due to our small sample of only eight species.

The data overall provide no evidence to support the idea that tuning properties of the auditory system change with, or in response to, call evolution in this clade. The data are most consistent with the idea that at this level of species relatedness auditory tuning is a phylogenetically conservative trait. Any call evolution that occurs does so in the face of this tuning stability, and therefore any match between tuning and call frequency (as in the case of the *P. pustulosus* chuck) is the result of changes in the call rather than in the receiver. Overall, we see very little evolution of AP tuning within the clade, and virtually none in

BP tuning. Body size is the strongest predictor of variation in BP tuning. But as most of these species are of similar size (table 1), differences in body size do not generate differences among species. Such body size differences can contribute to species or population-level differences in other anurans, however, by influencing BP tuning [Wilczynski, 1986; Keddy-Hector et al., 1992; McClelland et al., 1996, 1997]. Furthermore, work in cricket frogs indicates that size variation among individual females within populations and among populations within the species can affect female preferences, most likely through the effect on BP tuning [Ryan et al., 1992]. In this particular clade, however, any body size effect is too subtle, or the variation in body size within relative to that among populations is too great, to generate statistically significant differences among species.

We emphasize that our conclusions pertain strictly to evolution within the *Physalaemus pustulosus* species group. It is certainly the case that BP tuning varies greatly across anuran species [Zakon and Wilczynski, 1988; Gerhardt and Schwartz, 2001], including Neotropical species of a similar body size. For example, in *Eleutherodactylus coqui*, which is in the same family as *Physalaemus* (Leptodactylidae), males are slightly larger than túngara frogs but have a much higher BP BEF (37.2 mm snout-vent length (SVL), ca. 3,300 Hz), whereas *E. coqui* females are much larger than túngara frogs but have similar BP tuning (52.2 mm SVL; ca. 2,100 Hz) [Narins and Capranica 1976]. Two neotropical species, *Hyla ebraccata* and *H. microcephala*, both about the same size as túngara frogs, have BP tuning of ca. 2,800 and 5,400 Hz respectively [Wilczynski et al., 1993], with call dominant frequencies to match [McClelland et al., 1997]. Thus BP tuning has changed in frogs in general, although it is unknown whether the tuning change preceded or followed call changes or coevolved with them.

There may be greater selective pressure for better matches between tuning and call frequencies among closely related species with calls that are much simpler than the frequency modulated whines in the *P. pustulosus* species group. The *Physalaemus* whines all sweep through a considerable portion of the AP, and as such a close relationship between tuning sensitivity minima and call dominant frequency might not be critical. On the other hand, species such as *Hyla crucifer* or *H. microcephala*, which produce tonal calls [Brenowitz et al., 1984; McClelland et al., 1997], or *Acris crepitans,* which produces a series of short click-like calls with a single dominant frequency [Ryan and Wilczynski, 1988, 1991; Keddy-Hector et al., 1992; McClelland et al., 1996], might reveal a very differ-

ent pattern of call-tuning evolution. This is especially so as the calls of all of these species stimulate only the BP [Wilczynski et al., 1984, 1992, 1993], whose physiological properties suggest that it has very little capacity to accurately code complex frequency patterns [Zakon and Wilczynski, 1988].

Our results also pertain to only one parameter of tuning: the BEFs (or frequency at which threshold minima are located) of the two auditory end organs. It remains possible that other aspects of auditory processing change in response to changes in the communication system. These might include absolute thresholds of auditory fibers, the widths of the tuning curves, and central integration of information derived from the two end organs. First, we did not observe any obvious threshold differences, but this is due in part to variability in thresholds among electrode placements and across animals. The latter might be due to genuine differences in auditory physiology, but may also be the result of differences within and among species in, for example, their tolerance of captivity or anesthetic treatment. Second, precise quantification of tuning width, as with measurement of Q_{10} values, is problematic for multiunit data where the recorded signal is a combination of many different cells. An examination of such differences would be better addressed by a careful examination of tuning at the single unit level. Third, *P. coloradorum* females prefer their conspecific calls with appended *P. pustulosus* chucks over the normal call of their species. In part, this seems to be due to stimulation of the BP in *P. coloradorum*, which is not stimulated by the normal conspecific call. But this cannot entirely explain the preexisting preference because addition of chucks to the *P. enesefae* call, which should be stimulating the BP of this species as well (table 1), does not enhance call attractiveness [Tárano and Ryan, in review]. Thus the preexisting preference for chucks in the *P. pustulosus* clade might be based on stimulation of the BP as well as some aspects of acoustic processing in the central nervous system.

The best predictor of BP tuning was body size, rather than any particular call parameter. The significant relationship between body size and BP tuning among all frogs examined has been reported within several other species [Loftus-Hills, 1973; Shofner and Feng, 1981; Keddy-Hector et al., 1992; McClelland et al., 1996]. In contrast, the tuning of the AP is not related to body size. The difference between the two end organs in this regard is predicted by their mechanisms of tuning [Zakon and Wilczynski, 1988]. BP tuning is believed to result largely from mechanical tuning processes of the ear [Capranica and Mof-

fat, 1983; Lewis and Narins, 1999]. Ear components are allometrically related to body size, and, as they increase in size, their resonant properties shift to lower frequencies. One would then expect BP tuning to follow downward, yielding the negative correlation between size and tuning we found in this study and which others have reported. Tuning of the AP is no doubt partly related to mechanical tuning influenced by size, but it also appears to be influenced by the structural properties of the tectorial membrane [Lewis and Narins, 1999; Purgue and Narins, 2000] as well as electrical tuning inherent to the hair cells themselves [Lewis and Narins, 1999; Smotherman and Narins, 1999, 2000]. Neither of these would necessarily be expected to change with body size. As such, AP tuning changes have never been reported to correlate with body size, and we find no evidence of a strong relationship here.

Despite not using it for a communication function, the *Physalaemus* species examined in this study all retain a BP. The presence of this sensory channel that was not used for communication was critical in promoting sensory exploitation for the chuck. Thus it is of some interest to understand why such a 'functionless' organ might be maintained.

The most general and parsimonious explanation is that the BP, which is a primitive character for all amphibia, has fewer costs associated with maintaining it compared to what might be the costs and constraints of reorganizing the ear's developmental program to lose such an organ. For example, hair cell development in the AP might be genetically or mechanistically linked to BP hair cell development. Furthermore, the channels and fluid systems of the amphibian inner ear have recently been shown to be a very complicated mechanoacoustical system in which vibrations of different frequencies yield fluid displacement along different channels to the various contact and tectorial membranes associated with auditory transduction [Purgue and Narins, 2000]. It could be that the loss of any one chamber or channel would severely affect these fluid mechanics.

Moreover, it is hard to imagine an epigenetic mechanism such as disuse atrophy that could underlie the selective degeneration of the BP hair cells. Atrophy of sensory systems is usually related directly to lack of stimulation, or through a combination of disuse and selection for genetic changes in developmental programs leading to structural regression after some time of normal development. The best-studied examples are the reduced visual systems of cave fish [Wilkens, 1971; Voneida and Fish, 1984] and subterranean mammals [Burda et al., 1990].

Although the BP is not stimulated by conspecific calls in most members of the *Physalaemus pustulosus* species group, it would still be stimulated throughout life by ambient noise and heterospecific calls, which would thereby maintain activity in any hair cells that develop in either the AP or BP regardless of their use in detecting a conspecific call. Thus nonconspecific sounds could act as a constraint against the loss of one of these end organs. It is also important to note that there is evidence that even a small amount of stimulation, in the absence of any obvious ultimate utility, can be enough to maintain sensory capacity, as appears to be the case for the visual system of some subterranean mole-rats [Oelschläger et al., 2000].

Thus there appear to be constraints acting against frogs losing their inner ear organs. It is not known whether these constraints would be overridden if response to new signals represented a fitness cost [Ryan, 1985]; but there are no examples of any frog losing one of its auditory end organs. We suggest that game theory models that posit how evolution of communication systems should evolve could be improved by incorporating more explicit attention to some of these real biological issues in sensory evolution [cf. Bradbury and Vehrencamp, 2000].

It is also possible that the BP is retained without a communication function because it performs some other function. This might be the case in *P. pustulosus* [Phelps et al., in review]. These frogs cease calling after the approach of a frog eating bat, *Trachops cirrhosus* [Tuttle et al., 1982]. Latency to resume calling is reduced when *P. pustulosus* males hear conspecific calls or calls of the sympatric *Leptodactylus labialus*, but not calls of the allopatric *P. enesefae*. A typical *L. labialus* call sweeps from about 1,000 Hz to 2,500 Hz, and should mostly stimulate the BP of *P. pustulosus*. Thus retention of the BP despite lack of a communication function could be favored because of fitness benefits obtained by remote monitoring of predator presence. This is one example of what might be a general scenario; the auditory organ not used in conspecific communication might still be very useful in general hearing functions related to predator detection or monitoring other environmental disturbances [Wilczynski and Capranica, 1984]. In fact, Lewis [1987] suggested that the acoustical senses of the vertebrate ear arose originally to serve the function of remote detection of predators and prey. Nevertheless, demonstration of a non-communication function does not by itself prove that selection for this function, rather than the constraints outlined above, are responsible for maintenance of the BP.

The present results show that for all the *Physalaemus* species in this clade, BP tuning is statistically similar and hence the basic neurophysiological condition for sensory exploitation exists in the form of tuning that predates the emergence of the chuck that exploits it in one of the species, *Physalaemus pustulosus*. The present study investigated tuning largely in males, and therefore might best be regarded as showing that the use of the chuck in male-male communication results from a signal that evolved to exploit a pre-existing receiver sensitivity. An expanded study that incorporates an examination of potential BP (or AP) sex differences in each of the species is required before the entire situation can become clear. Nevertheless, in two of the investigated species, both males and females were examined, and there was no indication of any sex difference great enough to discount the conclusion that BP tuning in both sexes predates the evolution of the chuck.

References

Alexander, R.D. (1962) Evolutionary change in cricket acoustical communication. Evolution, *16:* 443–467.

Beeman, K. (1996) Signal. Engineering Design, Belmont, MA.

Boake, C.R.B. (1991) Coevolution of senders and receivers of sexual signals: Genetic coupling and genetic correlations. Trends Ecol. Evol., *6:* 225–231.

Borgia, G., and S.W. Coleman (2000) Co-option of male courtship signals from aggressive display in bowerbirds. Proc. R. Soc. Biol. Sci. B, *267:* 1735–1740.

Bradbury, J.W., and S.L. Vehrencamp (1998) Principles of Animal Communication. Sinauer Assoc. Inc., Sunderland, MA.

Bradbury, J.W., and S.L. Vehrencamp (2000) Economic models of animal communication. Anim. Behav., *59:* 259–268.

Brenowitz, E.A., W. Wilczynski, and H.H. Zakon (1984) Acoustic communication in spring peepers: Environmental and behavioral aspects. J. Comp. Physiol. A., *155:* 585–592.

Burda, H., V. Bruns, and M. Muller (1990) Sensory adaptations in subterranean mammals. Prog. Clin. Biol. Res., *335:* 269–293.

Cannatella, D.C., D.M. Hillis, P. Chippinendale, L. Weigt, A.S. Rand, and M.J. Ryan (1998) Phylogeny of frogs of the *Physalaemus pustulosus* species group, with an examination of data incongruence. Syst. Biol., *47:* 311–335.

Capranica, R.R. (1965) The Evoked Vocal Response of the Bullfrog: A Study of Communication by Sound. MIT Press, Cambridge, MA.

Capranica, R.R. (1966) Vocal response of the bullfrog to natural and synthetic mating calls. J. Acoust. Soc. Am., *40:* 1131–1139.

Capranica, R.R. (1968) The vocal repertoire of the bullfrog *(Rana catesbeiana).* Behaviour, *21:* 302–325.

Capranica, R.R., and A.J.M. Moffat (1980) Nonlinear properties of the peripheral auditory system of anurans. *In* Comparative Studies of Hearing in Vertebrates (ed. by A.N. Popper and R.R. Fay), Springer-Verlag, New York, pp. 139–165.

Capranica, R.R., and A.J.M. Moffat (1983) Neurobehavioral correlates of sound communication in anurans. *In* Advances in Vertebrate Neuroethology (ed. by J.-P. Ewert, R.R. Capranica and D. Ingle), Plenum Press, New York, pp. 701–730.

Carew, T.J. (2000) Behavioral Neurobiology, The Cellular Organization of Behavior. Sinauer Assoc. Inc., Sunderland, MA.

Doherty, J., and R.R. Hoy (1985) Communication in insects III. The auditory behavior of crickets: Some views of genetic coupling, song recognition, and predator detection. Quart. Rev. Biol., *60:* 453–472.

Endler, J.A. (1992) Signals, signal conditions, and the direction of evolution. Am. Nat., *139:* S125–S153.

Endler, J.A., and A.L. Basolo (1998) Sensory ecology, receiver biases and sexual selection. Trends Ecol. Evol., *13:* 415–420.

Felsenstein, J. (1985) Phylogenies and the comparative method. Am. Nat. *125:* 1–15.

Gerhardt, H.C., and J.J. Schwartz (2001) Auditory tuning and frequency preferences in anurans. *In* Anuran Communication (ed. by M.J. Ryan), Smithsonian Institution Press, Washington D.C., pp 73–85.

Hauser, M.D. (1996) The Evolution of Animal Communication. MIT Press, Cambridge, MA.

Hoy, R.R., J. Hahn, and R.C. Paul (1977) Hybrid cricket auditory behavior: Evidence for genetic coupling in animal communication. Science, *195:* 82–84.

Keddy-Hector, A.C., W. Wilczynski, and M.J. Ryan (1992) Call patterns and basilar papilla tuning in cricket frogs. II. Intrapopulational variation and allometry. Brain Behav. Evol., *39:* 238–246.

Lewis, E.R. (1984) On the frog amphibian papilla. Scan. Electron Microsc., *1984:* 1899–1913.

Lewis, E.R. (1987) Speculations about noise and the evolution of vertebrate hearing. Hear. Res., *25:* 83–90.

Lewis, E.R., and R.E. Lombard (1988) The amphibian inner ear. *In* The Evolution of the Amphibian Auditory System (ed. by B. Fritzsch, M.J. Ryan, W. Wilczynski, T.E. Hetherington and W. Walkowiak), Wiley, New York, pp. 93–123.

Lewis, E.R., and P.M. Narins (1999) The acoustic periphery of amphibians: Anatomy and physiology. *In* Comparative Hearing: Fish and Amphibians (ed. by R.R. Fay and A.N. Popper), Springer-Verlag, New York, pp. 101–154.

Lewis, E.R., E.I. Hecht and P.M. Narins (1992) Diversity of form in the amphibian papilla of Puerto Rican frogs. J. Comp. Physiol. A, *171:* 421–435.

Loftus-Hills, J.J. (1973) Comparative aspects of auditory function in Australian anurans. Aust. J. Zool., *21:* 353–367.

Martins, E.P. (2001) COMPARE, version 4.4. Computer programs for the statistical analysis of comparative data. Distributed by the author via the www at http://compare.bio.indiana. edu/. Department of Biology, Indiana University, Bloomington, IN.

Martins, E.P., and T.F. Hansen (1997) Phylogenies and the comparative method: A general approach to incorporating phylogenetic information into analysis of interspecific data. Am. Nat., *149:* 646–667.

McClelland, B.E., W. Wilczynski, and A.S. Rand (1997) Sexual dimorphism and species differences in the neurophysiology and morphology of the acoustic communication system of two neotropical hylids. J. Comp. Physiol. A., *180:* 451–462.

McClelland, B.E., W. Wilczynski, and M.J. Ryan (1996) Correlations between call characteristics and morphology in male cricket frogs *(Acris crepitans).* J. Exp Biol., *199:* 1907–1919.

Narins, P.M., and R.R. Capranica (1976) Sexual differences in the auditory system of the tree frog *Eleutherodactylus coqui.* Science, *192:* 378–380.

Oelschläger, H.H.A., M. Nakamura, M. Herzog, and H. Burda (2000) Visual system labeled by c-Fos immunohistochemistry after light exposure in the 'blind' subterranean Zambian mole rat *(Cryptomys anselli).* Brain Behav. Evol., *55:* 209–220.

Phelps, S.M., A.S. Rand, and M.J. Ryan (in review) Túngara frogs eavesdrop on heterospecific calls: The predation-rumor hypothesis and the function of a sensory bias. Anim. Behav.

Purgue, A.P., and P.M. Narins (2000) Mechanics of the inner ear of the bullfrog *(Rana catesbeiana):* the contact membranes and the periotic canal. J. Comp. Physiol. A., *186:* 481–488.

Ryan, M.J. (1980) Female mate choice in a neotropical frog. Science, *209:* 523–525.

Ryan, M.J. (1985) The Túngara Frog. A Study in Sexual Selection. University of Chicago Press, Chicago, IL.

Ryan, M.J. (1986) Neuroanatomy influences speciation rate in anurans. Proc. Nat. Acad. Sci., *83:* 1379–1382.

Ryan, M.J. (1990) Sexual selection, sensory systems, and sensory exploitation. Oxford Surv. Evol. Biol., *7:* 46–52.

Ryan, M.J. (1997) Sexual selection and mate choice. *In* Behavioral Ecology, an Evolutionary Approach, Fourth Edition (ed. by J.R. Krebs and N.R. Davis), Blackwell, Oxford, pp. 179–202.

Ryan, M.J. (1998) Receiver biases, sexual selection and the evolution of sex differences. Science, *281:* 1999–2003.

Ryan, M.J., and A.S. Rand (1993) Phylogenetic patterns of behavioral mate recognition systems in *Physalaemus pustulosus* species (Anura: Leptodactyllidae): the role of ancestral and derived characters and sensory exploitation. Linnean Soc. Symp. Ser., *14:* 251–267.

Ryan, M.J., and A.S. Rand (1995) Female responses to ancestral advertisement calls in the túngara frog. Science, *269:* 390–392.

Ryan, M.J., and A.S. Rand (1998) Evoked vocal response in male túngara frogs: Preexisting biases in male responses? Anim. Behav.*, 56:* 1509–1516.

Ryan, M.J., and A.S. Rand (1999) Phylogenetic inference and the evolution of communication in túngara frogs. *In* The Design of Animal Communication (ed. by M. Hauser and M. Konishi), MIT Press, Cambridge, Mass., pp. 535–557.

Ryan, M.J., and A.S. Rand (2001) Feature weighting in signal recognition and discrimination by túngara frogs. *In* Anuran Communication (ed. by M.J. Ryan), Smithsonian Institution Press, Washington D.C., pp. 86–101.

Ryan, M.J., and W. Wilczynski (1988) Coevolution of sender and receiver: Effect on local mate preferences in cricket frogs. Science, *240:* 1786–1788.

Ryan, M.J., and W. Wilczynski (1991) Evolution of intraspecific variation in the advertisement call of a cricket frog *(Acris crepitans,* Hylidae). Biol. J. Linn. Soc., *44:* 249–271.

Ryan, M.J., J.H. Fox, W. Wilczynski, and A.S. Rand (1990) Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus.* Nature, *343:* 66–67.

Ryan, M.J., S.A. Perrill, and W. Wilczynski (1992) Auditory tuning and call frequency predict population-based mating preferences in the cricket frog, *Acris crepitans.* Am. Nat., *139:* 1370–1383.

Shaw, K. (1995) Phylogenetic tests of the sensory exploitation model of sexual selection. Trends Ecol. Evol., *10:* 117–120.

Shofner, W.P., and A.S. Feng (1981) Post-metamorphic development of frequency selectivities and sensitivities of the peripheral auditory system of the bullfrog, *Rana catesbeiana.* J. Exp. Biol., *93:* 181–196.

Smotherman, M.S., and P.M. Narins (1999) The electrical properties of auditory hair cells in the frog amphibian papilla. J. Neurosci., *19:* 5275–5292.

Smotherman, M.S., and P.M. Narins (2000) Hair cells, hearing and hopping: A field guide to hair cell physiology in the frog. J. Exp. Biol., *203:* 2237–2246.

Tárano, Z., and M.J. Ryan (in review) No preexisting biases for heterospecific call traits in the frog *Physalaemus enesefae.* Anim. Behav.

Tuttle, M.D., L.K. Taft, and M.J. Ryan (1982) Evasive behaviour of a frog in response to bat predation. Anim. Behav., *30:* 393–397.

Voneida, T.J., and S.E. Fish (1984) Central nervous system changes related to the reduction of visual input in a naturally blind fish *(Astyanax hubbsi).* Am. Zool., *24:* 775–782.

Wever, E.G. (1985) The Amphibian Ear. Princeton University Press, Princeton, NJ.

Wilczynski, W. (1986) Sexual differences in neural tuning and their effect on active space. Brain Behav. Evol., *28:* 83–94.

Wilczynski, W., and R.R. Capranica (1984) The auditory system of anuran amphibians. Prog. Neurobiol., *22:* 1–38.

Wilczynski, W., A.C. Keddy-Hector, and M.J. Ryan (1992) Call patterns and basilar papilla tuning in cricket frogs. I. Differences among populations and between sexes. Brain Behav. Evol., *39:* 229–237.

Wilczynski, W., B.E. McClelland, and A.S. Rand (1993) Acoustic, auditory, and morphological divergence in three species of neotropical frog. J. Comp. Physiol. A, *172:* 425–438.

Wilczynski, W., A.S. Rand, and M.J. Ryan (1995) The processing of spectral cues by the call analysis system of the túngara frog, *Physalaemus pustulosus.* Anim. Behav., *49:* 911–929.

Wilczynski, W., A.S. Rand, and M.J. Ryan (1999) Female preferences for temporal order of call components in the túngara frog: a Bayesian analysis. Anim. Behav., *58:* 841–851.

Wilczynski, W., H.H. Zakon, and E.A. Brenowitz (1984) Acoustic communication in spring peepers. Call characteristics and neurophysiological aspects. J. Comp. Physiol. A., *155:* 577–584.

Wilkens, H. (1971) Genetic interpretation of regressive evolutionary processes: studies on hybrid eyes of two *Astyanax* populations (Characidae, Pices). Evolution, *25:* 530–544.

Wilkinson, L. (1996) Systat. SPSS Science Marketing Dept, Chicago, Ill.

Zakon, H.H., and W. Wilczynski (1988) The physiology of the anuran eighth nerve. *In* The Evolution of the Amphibian Auditory System (ed. by B. Fritzsch, M.J. Ryan, W. Wilczynski, T.E. Hetherington and W. Walkowiak), Wiley, New York, pp. 125–155.