

CORRELATIONS BETWEEN CALL CHARACTERISTICS AND MORPHOLOGY IN MALE CRICKET FROGS (*ACRIS CREPITANS*)

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Summary

We investigated the relationships among spectral and temporal advertisement-call characteristics and the sizes of the laryngeal and ear components thought to underlie the generation and reception of species-specific vocalizations in male cricket frogs (*Acris crepitans*). We tested the predictions that the volumes of the structural elements necessary for acoustic communication would be correlated with various parameters of the vocalizations. The anatomy of laryngeal and ear structures was reconstructed from serial sections of the heads of male cricket frogs of two subspecies collected from several sites across the range of this species in Texas, USA. The relationships among the anatomy and call parameters were assessed using several univariate and multivariate analyses. Highly significant univariate correlations among the laryngeal components suggest that the temporal and spectral characteristics of the

calls are not independently produced. Dominant frequency correlates strongly with most of the other call and morphological characteristics. Removing body size effects, however, removes the relationship between dominant frequency and the volume of the whole larynx and ear. This is also the case for call pulse rate, indicating that for this species both spectral and temporal call parameters are biomechanically related to laryngeal size which is, in turn, largely mediated by body size. General body size effects might also explain the existence of significant relationships between ear size and temporal characteristics of the call that probably do not have a functional basis.

Key words: larynx, ear, vocalization, anuran, cricket frog, *Acris crepitans*.

Introduction

Acoustic communication figures prominently in the social behavior of most anuran amphibians. Although the circumstances in which animals vocalize vary among species, virtually all male frogs incorporate some form of advertisement call into their vocal repertoire that is usually a necessary precursor to successful courtship and mating (Wells, 1988; Rand, 1988; Gerhardt, 1988). Each anuran species produces an advertisement call with unique temporal and spectral parameters, enabling females to identify and select conspecific males for mating. Although the acoustic characteristics of advertisement calls are species-specific, they can vary among populations and individuals (Capranica *et al.* 1973; Nevo and Capranica, 1985; Sullivan, 1985; Ryan and Wilczynski, 1988, 1991; Wagner, 1989*a,b*; Keddy-Hector *et al.* 1992; Wilczynski *et al.* 1992). It is the intraspecific diversity in call characteristics that allows females to discriminate among potential conspecific mates on the basis of some of the same acoustic parameters used for species identification (Ryan *et al.* 1992). Evolutionary or proximate factors, such as optimizing transmission clarity or attractiveness to females, have influenced the development of specific acoustic parameters of advertisement calls. Interspecific differences in the size and shape of anuran larynges suggest that these selection forces can

operate directly on the structure of the larynx, thus ensuring that each species produces unique vocalizations (McAlister, 1959, 1961; Schmidt, 1965*a,b*, 1973*a,b*, 1976).

Anuran vocalizations result from interactions between the morphological structure of the larynx and vocal tract (passive elements in the generation of sound) and movements of the arytenoid cartilages which modulate the airflow as it exits the lungs (Martin, 1971, 1972; Martin and Gans, 1972; Schneider, 1988). Audible sound is produced when the airflow causes the vocal cords to begin vibrating. The frequency of this vibration and the resulting vocalizations are influenced not only by the pressure of the airflow through the larynx but also by the size, tension and mass of the vocal cords (Martin, 1972). Additionally, movements of the laryngeal muscles, abdominal muscles and arytenoid valves, either passively due to the airflow or actively due to muscle contractions, create the temporal parameters of the calls by breaking the vibrations into pulses (Martin, 1971, 1972; Martin and Gans, 1972; Schmidt, 1972*a,b*; Gans, 1973). It has been hypothesized that sexually dimorphic laryngeal muscle size in *Rana pipiens* is responsible for temporal differences in release call characteristics. These vocalizations, which are produced by both males and females to extricate themselves from inappropriate sexual clasps, have

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a well-defined pulse structure and increased amplitude modulation in males with large laryngeal muscles compared to females with small laryngeal muscles (McClelland and Wilczynski, 1989a,b). Short advertisement calls are also associated with large laryngeal muscles in tropical hylid species, suggesting that greater muscle volume is an effective controlling force for pulse generation in these males (Wilczynski *et al.* 1993). Thus, in general, the frequency or spectral characteristics of anuran vocalizations are influenced by the morphology of the larynx and buccal cavity, whereas the temporal or pulsatile structure of a call is usually derived in part from movements of the larynx itself.

An efficient acoustic communication system relies not only on the accurate production of vocalizations but also on an auditory system specialized for the reception of conspecific calls. This coupling of signal and receiver has been well documented in anurans: both inter- (Zakon and Wilczynski, 1988) and intraspecific (Ryan and Wilczynski, 1988; Wilczynski *et al.* 1992) differences in calls are correlated with different physiological properties of the auditory system. The relationship between call characteristics and features of the anatomical structure of the peripheral auditory system, such as the size of middle or inner ear cavities, is not well documented, although some evidence indicates that structural features can influence the range of sounds detectable by an animal (Eggermont, 1988; Fox, 1995). For instance, the efficacy of an acoustic system in responding to species-specific calls is enhanced by an appropriate neural tuning of the receiver's auditory periphery, and the neural response, in turn, is influenced by the resonant properties of the middle and inner ear. It is possible that these structures act as bandpass filters and, as such, can optimize the sensitivity of the ear to a particular range of frequencies (Chung *et al.* 1978, 1981; Hetherington and Lombard, 1982). Therefore, one might predict a relationship between the size of the peripheral ear cavities and specific acoustic parameters of conspecific vocalizations, such as a possible inverse correlation with call dominant frequency. Correlations among temporal parameters of the vocalizations and the size of auditory structures are more difficult to predict, as previous studies addressing the detection of temporal acoustic variables in anurans have not included information that would suggest hypotheses about the relationship of these traits to peripheral morphology (Feng *et al.* 1990, 1991).

Although it is clear that the size of the larynx or ear might affect vocalization characteristics and auditory reception, it is unknown whether differences in laryngeal and ear size simply reflect allometric body size changes. Thus, the morphometric relationships among the larynx, ear, body size and vocalizations might provide some insight into the anatomical basis of specific call characteristics and auditory reception, while addressing questions regarding the general effect of body size on vocal communication in anurans.

One way to investigate relationships among the anatomical aspects of anuran acoustic communication and vocalizations is to focus on a wide-ranging species in which call characteristics demonstrate a significant level of intraspecific variation.

Cricket frogs (*Acris crepitans*) fulfill these criteria, and we examined the laryngeal and ear morphology of specimens from six populations that have previously been shown to have significant geographical variation in their spectral and temporal advertisement call characteristics (Nevo and Capranica, 1985; Ryan and Wilczynski, 1991). *Acris crepitans* is an abundant, non-arboreal hylid species, which occurs throughout the midwest of the USA and in most Texas counties. Potential species-wide relationships established between vocalization characteristics and laryngeal component sizes might suggest possible mechanisms of call production in this species, as well as morphological changes leading to call diversity within the species. Additionally, we examined the relationships between structural elements of the ear of the cricket frog and call characteristics that might suggest peripheral influences on the range of sounds detectable by the auditory system.

Materials and methods

We examined 59 male cricket frogs (*Acris crepitans* Baird) that had been collected as part of a larger survey and analysis of population variation in cricket frog call characteristics (see Ryan and Wilczynski, 1991). The animals were collected from six sites across Texas, USA, close to a transect at $30.5 \pm 0.5^\circ$ latitude and were shown in previous reports to have a significant level of individual and population variation in call characteristics (Ryan and Wilczynski, 1988, 1991). Therefore, we assumed that there was sufficient diversity to reveal morphological correlations with call parameters, if present. Measurements from the specimens were pooled across populations before analysis to ascertain species-wide relationships among the anatomical structures and call characteristics, rather than intraspecific variation by population or geographic site.

Advertisement call groups were recorded from individuals in the field on metal tape using either a Sony TCD-5M or a Marantz PMD 420 cassette tape recorder, and a Sennheiser ME 80 microphone with a K3U power module and MZW 415 windscreen. The water temperature at the calling site was recorded and the call variables were subsequently adjusted for differences in temperature according to equations derived by Wagner (1989a) for this species. These calling males were collected and killed by an overdose of anesthetic in an aqueous solution of MS-222. The animals were fixed in 10% formalin and transferred to a 70% ethyl alcohol solution for preservation. The snout-vent lengths (SVL) and head widths of the specimens were measured using Vernier calipers to the nearest 0.1 mm.

Fifteen advertisement calls from each animal were analyzed for temporal and spectral acoustic characteristics on a Data Precision 6000, model 610, waveform analyzer (0.2 ms temporal and 13–26 Hz spectral resolutions; sampling rate 10 kHz). Spectral information was obtained from a Fast Fourier Transform of a call and dominant frequency was defined as the frequency containing the greatest amount of energy. In the previous study (Ryan and Wilczynski, 1991), call dominant

frequencies did not vary significantly over the duration of the vocalization, so only one value was reported for each male.

Of the 14 acoustic parameters analyzed in the previous study, dominant frequency, call duration and the number of pulses in the calls were the only characteristics that consistently showed variation by population, habitat and/or subspecies (Ryan and Wilczynski, 1991); thus, we concentrated on these acoustic traits as those most likely to show correlations with morphological features. Additionally, we calculated pulse rate (pulses s^{-1}) for each individual from the measurements of duration and number of pulses. Cricket frog calls are grouped into species-typical patterns (Fig. 1) and show graded variation from the beginning to the end of a call group (Wagner, 1989b; Ryan and Wilczynski, 1991). The call duration, number of pulses and number of pulse groups increase from the beginning to the end of a call group, whereas pulse rate decreases (Wagner, 1989b). Wagner (1989a) showed that the more invariant calls at the beginning of a group serve primarily as an attractant to females and the more variable temporal parameters of the middle and later calls can be altered to reflect aggression level. Thus, we examined the less variable earlier portions of the call groups rather than the middle or later portions.

We processed the specimens so that serial sections showing the ear and larynx (Fig. 2) were obtained. We began the morphological procedures by dissecting the head and throat of each animal, removing as much skin as possible. We placed the heads in a decalcifying agent (Cal-Ex, Fisher Diagnostics) for 24–36 h, after which we dehydrated the specimens in ethyl alcohol and xylenes before embedding them in paraffin. We sliced the heads using a microtome at a thickness of 25 μm through the ear and laryngeal region and mounted all tissue slices on microscope slides that had been prepared by dipping in a weak gelatin solution. We stained the sections using Pollak's trichrome (Humason, 1972) and Cresyl Violet to differentiate the muscles and cartilage. We processed the tissues in batches from randomly selected individuals to avoid the confounding problem of inter-batch variation in the effects of dehydration and embedding procedures on morphological measurements.

We measured the laryngeal or auditory structures of interest by projecting the slide on a Summagraphic 2201 digitizing pad interfaced with an IBM PC-AT that had been programmed with Sigma-scan (version 3.0, Jandel Scientific) graphics software. We traced the perimeters of the relevant areas in every tenth section (i.e. at intervals of 250 μm). The ear structures measured included the middle and inner ear cavities, the extracolumella cartilages and the tympanic membranes. In the larynx, we measured the sizes of arytenoid cartilages, the vocal cords, the basal cartilage of the arytenoid and the laryngeal constrictor and dilator muscles. We subsequently calculated the volumes (V_{total}) of each anatomical structure from the area measurements of each slice using the formula:

$$V_{\text{total}} = \sum V_{\text{slice}} = \sum_{i=0}^{n+1} T \frac{\sqrt{(A_i)(A_{i+1})} + A_i + A_{i+1}}{3},$$

where i is a running index of the order of the measured slices,

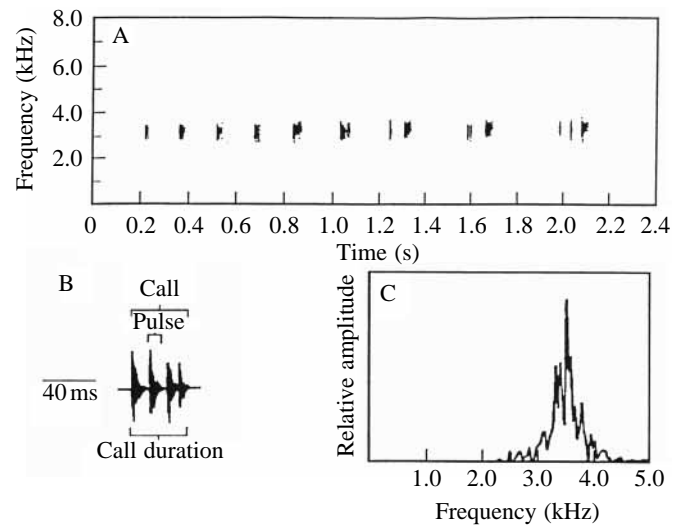


Fig. 1. A sonogram (A), oscillogram (B) and spectrogram (C) of a typical cricket frog (*Acris crepitans*) call group, call and pulses. The sonogram (A) shows a call group containing nine calls. The oscillogram (B) demonstrates the fine structure of a typical call, including the amplitude-modulated pulses. The spectrogram (C) is a Fast Fourier Transform of a single call with dominant frequency defined as the frequency containing the greatest amount of energy. Calls generally have different temporal properties depending on whether they are at the beginning, middle or end of a call group. The number of pulses in the calls can also vary with position in the call group. In general, *Acris crepitans* calls are longer and contain more pulses at the end of a call group, although the dominant frequency does not differ for the duration of the call group.

$i=1$ is the first slice with measurable area, n is the last slice with measurable area, A_i is the area of a structure (in mm^2) in one section, A_{i+1} is the area of the structure in the next measured section, and T is the distance between these slices (in this case, 250 μm). This formula for the volume of a frustum (V_{slice}) does not rely on the assumption that the area measurements for each slice are equal in order to calculate the volume accurately. We also measured the diameter of the tympanic membrane using a linear setting of the Sigma-scan program. Whenever possible, we made bilateral measurements, the values of which were consistently within 10% of each other. We averaged the resulting volumes or diameters to yield one value for each structure per animal.

We generated a correlation matrix of the measured call characteristics and morphological traits to examine general relationships among the variables across all individuals. Most anuran species, including *Acris crepitans*, show a strong relationship between body size and call characteristics, especially dominant frequency (Ryan, 1988; Zakon and Wilczynski, 1988). Thus, we determined whether body size differences can fully explain the variation observed in call characteristics or the underlying morphological components of the communication system. In order to examine the relationships among call parameters and morphological traits without the influence of body size, we used residuals from the regression of call or anatomical trait on SVL in a multivariate

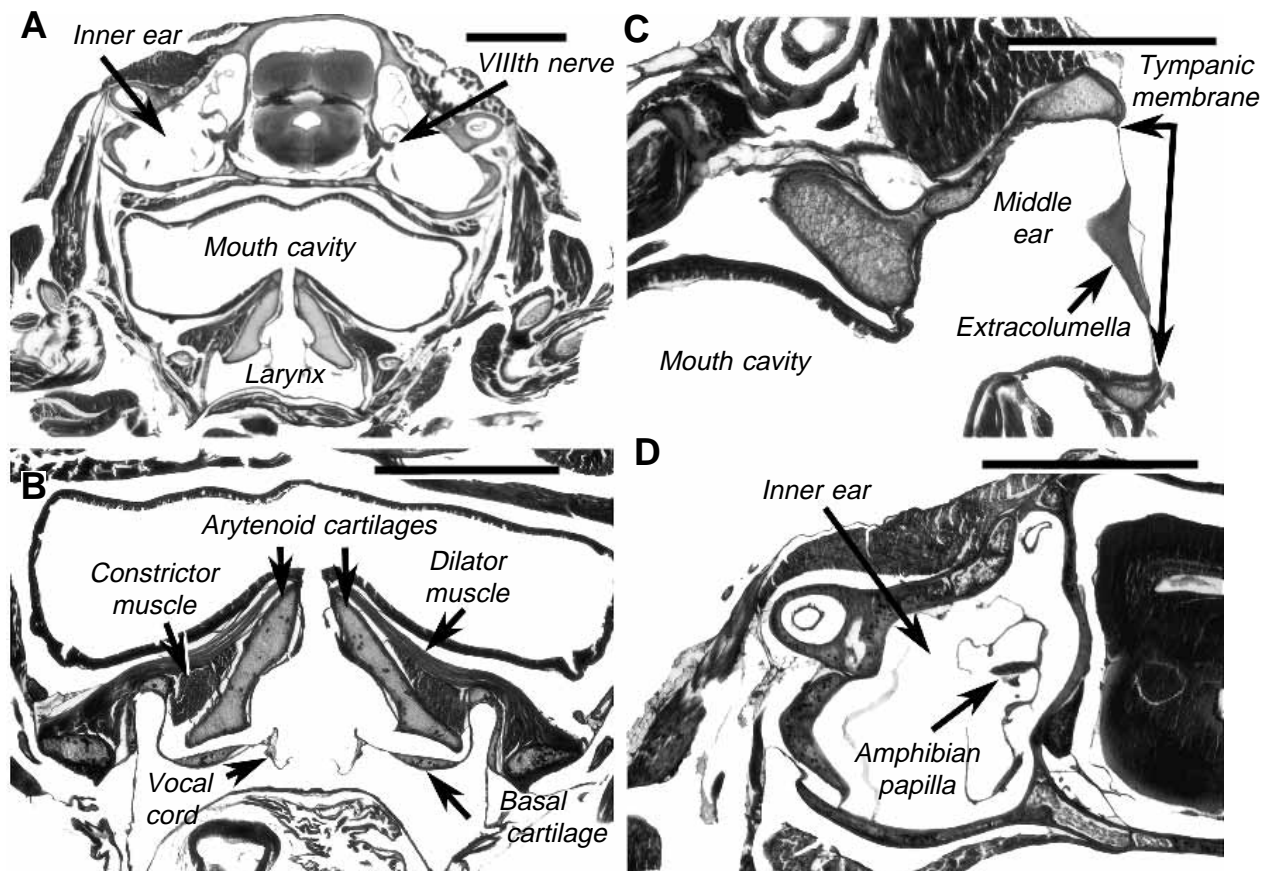


Fig. 2. Cross sections (25 μ m thick) through the whole head (A), larynx (B), middle ear (C) and inner ear (D) of a male cricket frog (*Acris crepitans*). Sound is produced by the larynx (A,B) when air is blown across the vocal cords through the glottal slit, setting up a fundamental frequency. Movements of the arytenoid cartilages create the temporal pulse structure of the call. Basal cartilages are characteristic of male hylids, but have an unknown function. Laryngeal components measured in this study included the volumes of the arytenoid cartilages, vocal cords, basal cartilages and constrictor and dilator muscles. The middle ear cavity (C) is located behind the tympanic membrane, which has an extracolumellar cartilage in its center. The inner ear (D) houses components of the peripheral auditory system, including the amphibian papilla, basilar papilla (not shown) and cranial nerve VIII. Auditory components measured in this study included the tympanic membrane diameter, the extracolumella volume and the volumes of the middle and inner ear cavities. Scale bars, 1.0 mm.

regression analysis analogous to the analyses using raw values. In all the data analyses, we report the conventional probability levels (i.e. $P \leq 0.05$ or $P \leq 0.01$) to demonstrate the significance of the results. We analyzed the data using a SYSTAT statistical package for the Macintosh (version 3.2; Systat Inc., Evanston, IL, USA). The means and range of values for the call and morphological characteristics of cricket frog males are shown in Table 1. Although the calls of 59 males were recorded and analyzed, we were unable to include all these samples in the morphological analysis due to attrition during the histological procedures which resulted in sample size differences for each variable as indicated in Table 1.

Results

Univariate correlations

Many vocal and anatomical characteristics covary, as demonstrated by the univariate correlation matrix of raw values

(see Materials and methods; Table 2). The correlation analysis of call parameters and anatomical characteristics indicates that some traits, such as SVL or dominant frequency are highly correlated with most other characteristics, whereas measurements such as tympanic membrane diameter or call duration only show relationships with two or three characteristics. Body size, as measured by SVL, is a particularly reliable morphological predictor of most vocal and anatomical characteristics in the male cricket frogs we sampled. SVL correlates with all the measured call and anatomical characteristics except call duration and tympanic membrane diameter. Correlations between morphological characteristics and SVL have positive values, indicating that the sizes of all the component parts increase with increasing body size. Standard coefficients (r -values) of the significant SVL correlations with morphological traits are high (range 0.466–0.809; Table 2), demonstrating that a considerable portion of the individual variation in ear and laryngeal structures can be explained by differences in body size. The

Table 1. *The range and mean values of male cricket frog (Acris crepitans) vocalization and morphological characteristics from Texas populations*

Characteristics	Range	Mean (\pm S.D.)
Vocalization characteristics		
Dominant frequency (kHz, $N=59$)	2.180–4.163	3.561 (\pm 0.448)
Call duration (ms, $N=59$)	11.822–46.558	29.421 (\pm 7.403)
Number of pulses in call ($N=59$)	2.474–7.672	4.980 (\pm 1.207)
Pulse rate (pulses per call, $N=59$)	85.453–256.365	175.280 (\pm 41.947)
Body and head size		
Snout–vent length (mm, $N=57$)	21.00–29.50	24.44 (\pm 2.26)
Head width (mm, $N=57$)	7.30–9.70	8.25 (\pm 0.55)
Laryngeal measurements		
Arytenoid cartilage volume (mm^3 , $N=52$)	0.420–0.988	0.699 (\pm 0.143)
Vocal cord volume (mm^3 , $N=50$)	0.031–0.076	0.051 (\pm 0.011)
Basal cartilage volume (mm^3 , $N=53$)	0.013–0.048	0.028 (\pm 0.007)
Constrictor muscle volume (mm^3 , $N=51$)	0.290–0.922	0.539 (\pm 0.138)
Dilator muscle volume (mm^3 , $N=52$)	0.105–0.462	0.199 (\pm 0.063)
Ear measurements		
Tympanic membrane diameter (mm, $N=56$)	0.881–1.330	1.058 (\pm 0.098)
Extracolumella volume (mm^3 , $N=56$)	0.008–0.035	0.021 (\pm 0.007)
Middle ear volume (mm^3 , $N=56$)	0.096–0.585	0.287 (\pm 0.103)
Inner ear volume (mm^3 , $N=56$)	0.479–1.749	1.207 (\pm 0.245)

significant univariate correlations between SVL and dominant frequency, number of pulses and pulse rate are negative.

Dominant frequency and temporal call characteristics exhibit a number of significant correlations with each other (Table 2). For example, dominant frequency is significantly correlated with call duration and pulse rate, but not with the number of pulses in the calls. High dominant frequency relates to shorter duration and faster pulse rates. Additionally, temporal parameters are correlated with each other in a logical manner. For example, longer calls contain more pulses, although the pulse rate is significantly slower in the longer calls.

Both spectral and temporal call characteristics are correlated with many morphological traits (Table 2). Dominant frequency, for example, is negatively correlated with all the anatomical components of the larynx and ear except tympanic membrane diameter. Temporal call characteristics, such as pulse rate, show some significant correlations with the laryngeal and ear measurements but, unlike call dominant frequency, do not show clear trends. In fact, the only morphological trait that significantly correlates with call duration is middle ear volume, and this is in a positive direction. The number of pulses in the calls shows a significant negative relationship with arytenoid cartilage, vocal cord and constrictor muscle volumes, but not with any measured morphological characteristics of the ear. Pulse rate is correlated with vocal cord, basal cartilage and constrictor muscle volumes, but not with arytenoid cartilage or dilator muscle volumes. Pulse rate is also the only spectral or temporal call characteristic that is significantly correlated with tympanic membrane diameter. Other correlations between pulse rate and ear morphology include the extracolumella and middle ear

volumes, which are both in a negative direction. Thus, in general, longer calls are associated with larger middle ear cavities, and faster pulse rates are associated with smaller extracolumella and middle ear volumes, but broader tympanic membranes.

In summary, significant univariate correlations among anatomical traits indicate that the sizes of laryngeal and ear components change together in a positive direction and all are strongly correlated with body size. Dominant frequency is inversely related to all the laryngeal and ear morphological characteristics except tympanic membrane diameter. Tympanic membrane diameter does not show the same basic pattern of relationships with the other morphological traits or call characteristics in that the only variable with which it significantly correlates is arytenoid cartilage volume. Temporal call characteristics show a more varied pattern of relationships with the morphological characteristics of the ear and larynx. Of the temporal characteristics measured, pulse rate most consistently shows significant univariate correlations with morphological characteristics.

Multiple regression analyses

Multiple regression analysis allows examination of the relationships among call characteristics and morphological characteristics of the whole larynx or ear, providing additional information in the form of partial correlation coefficients. In addition, by using the residuals of the regressions of body size (SVL) on call and morphological characteristics in the multiple regression analysis, the effects of body size are statistically removed from the calculations. Furthermore, by comparing these results with those from multivariate analyses using the raw values, the relative influence of body size on the

Table 2. Correlation matrix of male cricket frog (*Acris crepitans*) call and morphological characteristics

	Shout-vent length	Dominant frequency	Call duration	Number of pulses in call	Pulse rate	Arytenoid cartilage volume	Vocal cord volume	Basal cartilage volume	Constrictor muscle volume	Dilator muscle volume	Tympanic membrane diameter	Extra-columella volume	Middle ear volume	Inner ear volume
Dominant frequency	-0.827**	-												
Call duration	0.237	-0.339**	-											
Number of pulses in call	-0.360**	0.144	0.428**	-										
Pulse rate	-0.507**	0.421**	-0.579**	0.456**	-									
Arytenoid cartilage volume	0.672**	-0.547**	-0.003	-0.307*	-0.190	-								
Vocal cord volume	0.700**	-0.562**	0.086	-0.415**	-0.368**	0.684**	-							
Basal cartilage volume	0.546**	-0.561**	0.176	-0.218	-0.325*	0.500**	0.497**	-						
Constrictor muscle volume	0.749**	-0.647**	0.087	-0.537**	-0.506**	0.696**	0.682**	0.606**	-					
Dilator muscle volume	0.575**	-0.490**	0.123	-0.204	-0.240	0.687**	0.612**	0.537**	0.812**	-				
Tympanic membrane diameter	-0.018	-0.087	-0.157	0.173	0.340**	0.297*	0.075	0.246	0.013	0.262	-			
Extracolumella volume	0.466**	-0.412**	0.132	-0.183	-0.302*	0.392**	0.215*	0.287*	0.489**	0.299*	0.069	-		
Middle ear volume	0.809**	-0.693**	0.277*	-0.171	-0.383**	0.627**	0.613**	0.649**	0.638**	0.568**	0.115	0.350**	-	
Inner ear volume	0.596**	-0.562**	0.200	0.083	-0.097	0.483**	0.556**	0.439**	0.413**	0.435**	0.195	0.399**	0.665**	-

N=59; Pearson's product-moment r; *P<0.05; **P<0.01.

Table 3. Results from eight multiple regression analyses (multiple r and standardized coefficients of regression) on raw values of cricket frog call characteristics with morphological characteristics of the larynx and ear

	Dominant frequency	Call duration	Number of pulses in call	Pulse rate
Larynx	0.710**	0.302	0.563**	0.591**
Arytenoid cartilage volume	-0.084	-0.266	0.026	0.333
Vocal cord volume	-0.132	-0.023	-0.281	-0.193
Basal cartilage volume	-0.236	0.250	0.160	-0.115
Constrictor muscle volume	-0.358	0.232	-0.507*	-0.732**
Dilator muscle volume	-0.017	-0.032	0.085	0.226
Ear	0.723**	0.341	0.402	0.612**
Tympanic membrane diameter	0.014	-0.200	0.156	0.363**
Extracolumella volume	-0.168	0.035	-0.218	-0.257*
Middle ear volume	-0.133	0.060	0.385*	0.284
Inner ear volume	-0.548**	0.247	-0.369*	-0.523**

The results of the multiple regression are in bold type, other values are the partial regression coefficients of the individual traits.
* $P < 0.05$; ** $P < 0.01$.

Table 4. Results from eight multiple regression analyses (multiple r and standardized coefficients of regression) on residuals from the regression of call and morphological characteristics on body size (snout-vent length)

	Dominant frequency	Call duration	Number of pulses in call	Pulse rate
Larynx	0.238	0.309	0.389	0.432
Arytenoid cartilage volume	-0.108	0.395	-0.061	-0.532*
Vocal cord volume	-0.142	0.149	0.304	0.106
Basal cartilage volume	0.285	-0.327	-0.296	0.174
Constrictor muscle volume	0.152	-0.038	0.501*	0.539*
Dilator muscle volume	-0.044	-0.135	-0.346	-0.252
Ear	0.186	0.203	0.393	0.188
Tympanic membrane diameter	-0.324	0.193	0.400	0.110
Extracolumella volume	0.122	-0.110	0.173	0.367
Middle ear volume	0.137	-0.260	-0.098	0.188
Inner ear volume	0.151	0.051	-0.597*	-0.634*

The results of the multiple regression are in bold type, other values are the partial regression coefficients of the individual traits.
* $P < 0.05$; ** $P < 0.01$.

relationships between call characteristics and the peripheral laryngeal or auditory anatomy can be demonstrated.

When the individual laryngeal variables measured in this study are used in a multiple regression analysis, it is clear that the combined laryngeal traits are strongly related to most call characteristics including call dominant frequency, the number of pulses in the calls and the pulse rate (Table 3). When the same multiple regression analysis is performed on the residuals of the regressions of each characteristic on SVL (Table 4), the combined laryngeal traits are not significantly related to any of the call characteristics, indicating that body size accounts for much of the variation in call parameters. Partial regression coefficients show that, individually, none of the volumes of the component parts of the larynx significantly relates to call dominant frequency either with or without body size

influences. Partial regression coefficients do show that volumes of some laryngeal components are related to the temporal call parameters. For example, in the analysis of raw values with no body size correction, larger constrictor muscles are related to fewer pulses per call and a slower call rate (Table 3). Constrictor muscle volume is also related to these temporal call parameters after the effects of body size are removed, but the direction of the relationship is reversed (Table 4). Thus, after body size correction, larger constrictor muscles and smaller arytenoid cartilages are significantly correlated with a faster pulse rate.

In the multiple regression analysis using raw values, the combined ear traits are related to both dominant frequency and pulse rate (Table 3). Partial regression coefficients indicate that larger inner ear volumes are significantly related to calls

of lower dominant frequency, but that this relationship is non-significant after correction for body size (Table 4). With regard to relationships with the temporal call characteristics, large inner ear volumes correlate with fewer pulses in the calls and a slower pulse rate both before and after correction for general body size effects (Tables 3, 4). Other relationships among ear morphology and temporal characteristics are demonstrated by the results of the analysis on the raw values (Table 3). Middle ear volume is positively related to the number of pulses in the call such that fewer pulses relate to smaller middle ears. Additionally, lower pulse rates are associated with smaller tympanic membranes and larger extracolumella volumes.

Discussion

One difficulty in interpreting the results of our analyses is a general lack of information regarding the function of the anuran larynx. In this report, we concentrate on the correlations of laryngeal or ear size with call characteristics derived from the biomechanics of call generation. Because the acoustic characteristics of the cricket frog calls (Fig. 1) resemble those of Type I (Martin, 1972) calls of genus *Bufo*, we proceed below under the assumption that the vocal mechanisms of *Acris crepitans* are similar to those of *Bufo* even though they are not phylogenetically close to the bufonids. The Type I *Bufo* calls are generated by passive movements of the arytenoid cartilages rather than by active movements of the cartilages under direct muscle control.

Laryngeal morphology

The anatomical components of the larynx show strong intrastructural positive correlations, as would be expected if both spectral and temporal call parameters originate in the larynx. Univariate analyses also show that, among call characteristics, some temporal parameters (e.g. call duration and pulse rate) are correlated with dominant frequency (Table 2). The univariate analyses also demonstrate some predictable correlations among call and laryngeal morphological components. An inverse relationship between dominant frequency and the volumes of laryngeal components, especially that of the vocal cords, was expected, on the basis of the results of several previous reports. For example, differences in vocal cord length are clearly the principal source of differences between the fundamental frequencies of advertisement calls in the closely related similarly sized species *Bombina bombina* and *Bombina variegata* (Martin, 1972; Schmid, 1977). Conversely, vocal cords of the same volume in male and female *Rana pipiens* of the same body size are associated with calls of the same dominant frequency, despite volumetric differences in the other laryngeal components (McClelland and Wilczynski, 1989a,b). The presence of fibrous masses anchored dorsally deep within the bronchial processes of *Physalaemus pustulosus* larynges enable males to produce a complex call with an unexpectedly low frequency (Ryan, 1988; Ryan and Drewes, 1990), confirming a connection between structural size and call dominant frequency in anuran larynges.

Basal cartilage volume and other laryngeal variables are inversely related to dominant frequency in the univariate correlation matrix (Table 2). It has been suggested (Martin, 1971; Ryan and Drewes, 1990) that basal cartilages might influence the dominant frequency by increasing the volume and mass of vibratory tissue in the larynx. The additional mass attached to the vocal cords might lower the fundamental frequency of the larynx in a manner analogous to the fibrous masses of *Bufo* sp. or *Physalaemus pustulosus* (Martin, 1971; Ryan and Drewes, 1990). Variation in the sizes of other laryngeal components might have a similar effect.

A significant relationship between the combined laryngeal variables and dominant frequency in the multivariate analysis (Table 3) is consistent with this interpretation. None of the partial regression coefficients of the laryngeal variables, including vocal cord volume on dominant frequency, is statistically significant in the multiple regression models, perhaps because the component parts of the larynx are all highly interdependent. Thus, there appears not to be enough independent variance in any single variable to generate a significant partial regression coefficient.

When controlling for body size, neither the relationship between the combined laryngeal variables and dominant frequency nor any of the partial regression coefficients with dominant frequency was significant. Thus, a general increase in the size of an individual (as indicated by snout-vent length) allometrically increases the sizes of all laryngeal structures and thus decreases dominant frequency. Previous studies suggest that the vocal cords and possibly the basal cartilages are the structures most likely to be responsible for the determination of call frequency, but these and all other laryngeal components would allometrically increase or decrease together (Martin, 1972; Schmid, 1977).

Larger larynges result in lower call dominant frequencies owing to the acoustic relationship between size and vibration frequency. Larger larynges can also predict some temporal call parameters, such as lower pulse rate, because of the slower movements made by larger structures. In addition, the size of specific laryngeal components might also influence temporal parameters due to the biomechanics of call generation. Cricket frogs and other species with putative Type I passive vocalization mechanisms require a continuous positive pressure on the pulmonary air supply to sustain vocalizations and the pulse structure of the call. Calls in Type I species are terminated by dilator muscle contractions that separate the arytenoid cartilages, thus removing the vocal cords from the airstream and effectively terminating audible sound production. Although call duration is ultimately limited by lung capacity, there is a functional relationship between this temporal characteristic and the dilator muscles (Martin, 1972). The results of this study do not show a correlation between call duration and any of the measured laryngeal characteristics either in the univariate or in the multivariate analyses, regardless of body size influences. It is possible that the laryngeal muscles do control call duration, but that muscle size variation is not related to call duration in a way that would be

revealed by our study. Alternatively, the lack of correlation between call duration and the volumes of any laryngeal components might also suggest that in cricket frogs the larynx serves simply as a conduit for airflow. It is the airflow that creates audible sound by engaging laryngeal elements, but the duration of the call might be controlled by structures outside the larynx such as buccal or abdominal muscle contractions (Gans, 1974). This is the mechanism used to control duration in Types II and III calls, in which the lungs can be reinflated during a vocalized pulse train to sustain the sound by alternating compression of the buccal cavity and contractions of the abdominal muscles (Gans, 1974). If this is the mechanism for controlling call duration employed by cricket frogs, it represents a departure from a strictly Type I passive vocal production mechanism. It also suggests that a relationship between call duration and some other anatomical attribute, such as trunk or buccal muscle size, might exist. In this study, body size, as measured by SVL (a rough estimate of trunk volume), does not correlate with call duration. Perhaps a better measurement with which to assess possible anatomical factors controlling call duration would be trunk muscle thickness or another characteristic of the respiratory system.

Pulse generation, unlike call duration, is a temporal characteristic that clearly originates in the movements of the larynx. Laryngeal muscles are uniquely adapted to generate the rapid movements associated with the formation of a pulse structure in anuran vocalizations (Martin, 1971, 1972; Martin and Gans, 1972). It is logical that, in species with active pulse formation (call Types II and III), laryngeal muscle size could influence temporal parameters by affecting the power with which contractions occur, thereby affecting the movement of the arytenoid cartilages during pulse formation. Even in species with passive (Type I) amplitude modulation, laryngeal muscle size, as an index of fiber number and potential contraction power (Keynes and Aidley, 1991; Schneck, 1992), could possibly influence pulse characteristics (Schmidt, 1965*a,b*, 1976). For example, larger laryngeal muscles might regulate the rate of arytenoid cartilage movement. In this way, muscle size could affect the temporal properties of the call, resulting, for example, in faster pulses or deeper amplitude modulation.

In our study, partial regression coefficients from the multiple regression analyses show that constrictor muscle volume is significantly related to pulse rate and number of pulses in the calls both for the analysis of raw values and after correcting for body size effects (Tables 3, 4). This suggests that constrictor muscle size might serve as an agent, or at least a contributing factor, of variation in these temporal characteristics, as suggested by previous biomechanical studies. However, the trends of the relationships between constrictor muscle volume and temporal call characteristics are negative in the raw value multiple regression analysis and univariate correlation matrix, but positive after correction for body size, which creates a dilemma when interpreting the results even on a speculative basis. Contractions of the constrictor muscle are not responsible for pulse formation in

Type I vocalizations, but biomechanical evidence suggests that, together with the dilator muscles, these muscles enable the larynx to return to its prevocal state following a call (Martin and Gans, 1972; Schmidt, 1972*a,b*; Weber, 1976, 1977). Constrictor muscles also hold the glottal slit closed between Type I calls, while pulmonary air pressure increases in the larynx. It is the relaxation (rather than contraction) of the constrictor muscles and the subsequent explosive opening of the larynx that results in the pulse patterns characteristic of Type I calls (Martin, 1972; Martin and Gans, 1972). Thus, larger constrictor muscles might enable the arytenoid cartilages to sustain closure more effectively while the air pressure increases in the laryngeal lumen. In this speculative scenario, larger muscles could result in fewer pulses per call and a slower pulse rate. This explanation is supported in this study by the significant univariate correlations and by the partial regression coefficients using raw score values (Tables 2, 3), although not in the analysis after correcting for the effects of body size (Table 4).

The significant relationships of laryngeal morphology and call characteristics with body size in the univariate correlation matrix (Table 2) and, conversely, the lack of significant partial regression coefficients between most laryngeal components and call characteristics when the general effects of body size are removed (Table 4) suggest that a large portion of the variance among individuals within a species in the larynx and the calls it produces is due simply to the passive effects of general body growth. This has implications for the evolution of acoustic communication in anurans. It is possible that ecological or other pressures that act within a species to influence body size cannot do so without inadvertently altering the acoustic characteristics of the vocalizations. This may be particularly important in wide-ranging anuran species that occupy a variety of habitats, including arid environments which tend to favor animals with larger body sizes (Nevo, 1973; Nevo and Capranica, 1985). This is not to say that all call variation within a species is due simply to variation in body size. In fact, in *Acris crepitans*, there is significant call variation among populations in some parameters independent of body size (Ryan and Wilczynski, 1991). We are currently investigating how this relates to population-level variation in laryngeal structure. Nevertheless, our results indicate (1) that any change in body size will significantly affect call characteristics; (2) that body size changes will affect both spectral and temporal call characteristics; and (3) that the intercorrelation between all laryngeal components and consequently between spectral and temporal call characteristics suggests that any change along one dimension (e.g. spectral) might necessarily generate a change along another (e.g. temporal).

Ear morphology

Variation in the anatomy of the structures used for acoustic communication was also found in the ear morphology of cricket frogs. Because the ear is, in part, a mechanical resonator, changes in the size of its structures and cavities

could influence its tuning to spectral features of acoustic signals (Loftus-Hills, 1973; Eggermont, 1988). Furthermore, if the ear has a positive allometric relationship with an animal's body size, then larger animals might not only produce lower frequency calls, but might also have larger ears that are tuned to lower frequencies. A neurophysiological study of basilar-papilla tuning in two cricket frog populations (Keddy-Hector *et al.* 1992; Wilczynski *et al.* 1992) has shown that the best excitatory frequency of the eighth cranial nerve fibers that innervate the ear is negatively correlated with body size. Our morphological data suggest allometric changes in the ear that might be responsible for this effect and the relationship of those changes to call frequencies.

Univariate correlations show that all ear components, with the exception of tympanic membrane diameter, are positively correlated with body size and negatively correlated with call dominant frequency (Table 2). The exception for tympanic membrane diameter is consistent with a previous examination of this structure in cricket frogs. Ryan and Wilczynski (1991), in an extensive survey of population variation in cricket frog calls and body morphology, found that tympanic membrane diameter was more variable than SVL or head width and was the only morphological character that did not follow the same pattern of clinal variation in size as other body measurements. Their results suggest a higher degree of independence between tympanic membrane diameter and body size than found for other structures, which is confirmed by the observation that tympanic membrane diameter was the only ear variable that did not correlate with SVL in the present study. The variable nature of tympanic membrane diameter, plus the fact that we took individuals from several populations for this study, probably explains why there was no statistically significant correlation in our sample of frogs.

As predicted from the univariate analysis (Table 2), the relationship between the combined variables for the ear and call dominant frequency (Table 3) is significant. Previous studies indicate that middle ear cavity volume and tympanic membrane diameter should increase or decrease in size to 'match' the spectral parameters of the call (Eggermont, 1988). The univariate correlation matrix (Table 2) in our study shows a significant correlation between middle ear cavity volume and call dominant frequency, although the partial regression coefficient (Table 3) for middle ear volume shows that this ear structure does not individually relate to a significant amount of the variation in call dominant frequency, even when the effects of body size are corrected. In cricket frogs, tympanic membrane diameter also does not significantly correlate with call dominant frequency either in the univariate or in multivariate analyses, nor is there the same mathematical relationship between these characteristics as is seen in other species (Eggermont, 1988).

Male *Acris crepitans* middle ear volume shows a univariate correlation with dominant frequency (Table 2), but is not algebraically related to this spectral acoustic parameter in the same way as reported for other species (Hetherington and Lombard, 1982). For example, ranids have middle-ear resonant

frequencies lower than the call dominant frequency (Hetherington and Lombard, 1982), which was considered to be evidence for the transmission of non-tympanic frequencies by direct pressure pulses on the inner ear *via* the middle ear cavity. Large Eustachian tubes and the contiguous mouth cavity might also play a role in creating an optimal resonance chamber for the reception of vocalizations (Chung *et al.* 1978, 1981; Pinder and Palmer, 1983). The role (if any exists) of the mouth cavity in enhancing or limiting the reception of sound in anurans is still in dispute as there is evidence that middle ear resonance frequency is not affected by mouth position (Moffat and Capranica, 1978).

Our results do not show a significant relationship between tympanic membrane diameter and call dominant frequency and the evidence demonstrating a relationship between these two variables is not consistent. For example, during postmetamorphic growth in bullfrogs, *Rana catesbeiana*, tympanic membrane diameter increases tenfold and there is a correlated change in the best excitatory frequencies of the auditory neurons; specifically, a downward shift in frequency distribution as diameter increases (Shofner and Feng, 1981; Shofner, 1988). Eggermont (1988) also demonstrated a similar general relationship among several anuran species. Conversely, an interspecific survey showed no relationship between tympanic membrane area and frequency tuning of the ear (Fox, 1995). Fox's (1995) interspecific comparisons and a recent re-examination of tympanic membrane diameter variation in bullfrogs (Boatright-Horowitz and Megela Simmons, 1995) suggest instead a match between tympanic membrane diameter or area and auditory thresholds. Thus, it is not clear whether a match should be predicted between call dominant frequency and tympanic membrane diameter. Diameter, however, is not the only attribute that can affect the resonant frequency of the tympanic membrane and consequently the characteristics of the bandpass filter, as resonant frequency is also highly influenced by the tension, stiffness and mass of the tissue. For instance, a pad of extra mass in the center of a circular membrane can affect resonance properties much more profoundly than the same mass of material evenly distributed on the membrane surface (Powell and Roberts, 1923; Martin, 1972). This suggests a function for the extracolumella cartilage located near the center of the tympanic membrane. It is possible that the extracolumella volume could have more influence on the optimal frequency of vibration than the size of the tympanic membrane itself. The negative univariate correlation of extracolumella volume with dominant frequency (Table 2) supports this hypothesis and suggests that the extracolumella might serve in a compensatory manner, mediating the functional relationship between tympanic membrane diameter and call dominant frequency. Neither the tympanic membrane diameter nor the volume of the extracolumellar tissue by itself fully explains the variation in call dominant frequency found in our analysis.

Similar to the results from the laryngeal analyses, the sizes of the ear structures are strongly influenced by body size, and significant correlations between the ear structure variables and

call dominant frequency disappear when body size is statistically removed in the multivariate analysis (Tables 3, 4). Therefore, in cricket frogs, changes in body size significantly influence the size of the call-production apparatus and the calls it produces, and at the same time influence the match between these calls and the auditory reception system.

Correlations between ear component size and temporal call characteristics are more difficult to explain, and it is possible that these relationships, although statistically significant, do not have a functional basis. The eighth nerve encodes temporal parameters by a discharge pattern that responds as an 'envelope detector' (Feng *et al.* 1990), and it is difficult to derive a scenario in which the size of any ear structures could influence this process. Thus, it is unlikely that the sizes of ear structures in this species have evolved to maximize the accuracy of transmitting temporal call characteristics. It is more likely that the statistically significant relationships established between ear morphology and temporal characteristics are a coincidental by-product of the relationship between spectral and temporal call characteristics and the correlation of both characteristics and all morphological structures with body size. Thus, laryngeal volume changes will directly or indirectly induce changes in temporal call characteristics and, mediated through a similar relationship with body size, ear component size will also reflect these changes, resulting in statistically significant, but functionally ungrounded, relationships between ear component size and temporal call characteristics.

General conclusions

This study demonstrates that, in cricket frogs, general effects of body size have a strong influence on the size of all laryngeal components, and, because of this, on both spectral and temporal call characteristics. Our study also suggests that temporal and spectral call characteristics are not completely independent parameters, but are related because the laryngeal components responsible for these different acoustic parameters are found in the same anatomical structures and because all such components are influenced by the intervening variable of body size. Because of this, selection pressures favoring one call parameter might inadvertently affect another parameter. For example, possible sexual selection pressures to decrease call dominant frequency (Ryan *et al.* 1992) might result in larger vocal cords but, because associated muscles and cartilages would also be enlarged, the initial changes would also indirectly affect the call pulse rate.

These observations are significant because the final acoustic structure of the call and laryngeal morphology represent a compromise among various selection pressures not only to optimize transmission clarity in specific habitats but also to enhance the attractiveness of the call to females. Perhaps the fibrous masses or changes in muscle size found in the larynges of some anurans (e.g. *Physalaemus pustulosus*, Ryan and Drewes, 1990; *Hyla ebraccata*, Wilczynski *et al.* 1993) represent strategies to biomechanically uncouple those parts of the larynx responsible for different acoustic characteristics. This study suggests that, in cricket frogs, and perhaps within

anuran species in general, the relationships among laryngeal components and their mutual relationship to body size could constrain potential variation in call characteristics, regardless of the strength of the different selection pressures.

Changes in the larynx can affect vocalizations, influence the divergence of call characteristics among populations and, *via* the role of advertisement vocalizations in mate selection, potentially affect the course of speciation events. This study provides an opportunity to examine the correlated changes between call characteristics and morphology within a species and in so doing suggests ways that vocal mechanics might be operating in this species. Variation in vocal cord and basal cartilage volume is the probable determinant of variation in dominant frequency, although contributions from other structures cannot be discounted by our results. On the basis of vocal mechanisms established in other genera, it appears that pulses are produced passively by arytenoid cartilage movements initiated by airflow from the lungs that causes the vocal cords to vibrate (Martin, 1971, 1972; Martin and Gans, 1972). Laryngeal muscles possibly contribute to pulse formation by controlling the power with which the glottal slit is held closed against air pressure at the initiation of a call and the rate at which the arytenoid cartilages are returned to the prevocal configuration between pulses. This proposed laryngeal mechanism is consistent with the results of our study that show a positive correlation between constrictor muscle volume and the number of pulses in a call and increased pulse rate when the general effects of body size are removed.

Logical predictions can be made regarding the relationship between peripheral ear size and the optimal reception of certain frequencies. Cricket frogs conform to some of these predictions, in particular that the volumes of the extracolumella cartilage and middle and inner ear cavities are negatively correlated with call dominant frequency. Tympanic membrane diameter does not show a similar significant correlation with this spectral parameter. The significant correlations observed between temporal characteristics of calls and peripheral auditory anatomy are difficult to explain. These correlations might have a functional significance, although it is also possible that some of the correlations between temporal call characteristics and peripheral auditory anatomy emerge as a result of the effect of body size on both temporal and spectral call characteristics and are functionally inexplicable when considered in isolation.

It is noteworthy that both the ear structures and the laryngeal components are strongly influenced by the general effects of body size. Just as all laryngeal components covary with body size, leading to correlated changes in spectral and temporal call characteristics, the coincident size changes in the laryngeal and ear structures lead to correlated changes in the production and reception of calls. This is probably most important in maintaining their relationship in the spectral domain, but spectral cues, notably dominant frequency, are very important determinants of mate choice in cricket frogs (Ryan *et al.* 1992) and many other anuran species (Gerhardt, 1988; Ryan, 1988). It might certainly be the case that ears and larynges maintain

some degree of independence from each other. However, the constraints placed jointly on the larynx and ear by body size influences might place some limitations on the ability of these two components of the anuran communication system to diverge within a species.

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