Biological Journal of the Linnean Society (1998), *63:* 51–67. With 4 figures

Intraspecific variation in laryngeal and ear morphology in male cricket frogs (*Acris crepitans*)

BLINDA E. McCLELLAND[1], WALTER WILCZYNSKI[1,2] AND
MICHAEL J. RYAN[2]

Departments of [1]Psychology and [2]Zoology, The University of Texas, Austin, Texas 78712, U.S.A.

Received 22 February 1997; accepted for publication 22 August 1997

In a previous report, the authors found significant population variation in the calls of cricket frogs (*Acris crepitans*) that could not be explained by geographic variation in body size alone. Here we extend that work by investigating intraspecific population variation in the morphological characteristics underlying acoustic communication in male cricket frogs from several sites in Texas. We measured the volumes of laryngeal and auditory components responsible for the generation or reception of species-specific vocalizations in male frogs from eight populations. We found significant differences among populations in body size, as well as all the laryngeal and ear components we measured. With the exception of vocal cord and extracolumella volumes, the volumes of these anatomical structures differ among populations independently of body size as determined by a covariate analysis with snout-vent length as the covariate. Call dominant frequency differs among populations in a clinal pattern and head width, arytenoid cartilage, vocal cord and dilator muscle volume show a similar pattern when the residuals of the regression of morphological component on SVL are assessed for this trend. The results show that both larynx and ear structures can change in size independently of body size, yielding significant geographic variation in the behavioral and physiological expressions of the acoustic communication system underlying mate choice.

ADDITIONAL KEY WORDS:—larynx – ear – auditory – vocalizations – *Acris crepitans* – geographic variation – communication.

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Correspondence to: Blinda E. McClelland, Mezes Hall 330, Department of Psychology, University of Texas, Austin, TX 78712-1189 U.S.A., E-mail: mcclelland@mail.utexas.edu

0024–4066/98/010051 + 17 $25.00/0/bj970176

INTRODUCTION

A common feature of most anuran courtship systems is the production of species-specific advertisement calls by males. Although there has been considerable debate addressing whether the divergence in signals among taxa is a cause or a consequence of speciation events (Dobzhansky, 1937; Mayr, 1942; Lande, 1981; West-Eberhard, 1983; Andersson, 1994), evidence from behavioral and neuroethological studies indicate that the acoustic characteristics of anuran advertisement calls are diverse enough among species to enable accurate species discrimination while species integrity is maintained (Blair, 1958, 1974; Littlejohn, 1959, 1981, 1988; Mayr, 1963; Littlejohn & Watson, 1974; Gerhardt, 1974, 1982, 1988, 1994; Walkowiak, 1988; Zakon & Wilczynski, 1988; Rand, 1988). These calls operate interspecifically to signal a male's species, location and reproductive state while simultaneously performing an intraspecific function to repel competitor males and provide cues for females to choose a mate among the available conspecific males.

Given the negative consequences of incorrect mate decisions, it follows that the characteristics used to communicate species identity would be evolutionarily conservative (Passmore, 1981; Paterson 1985, 1993). Preferential mating for conspecifics exhibiting a limited set of acoustic characteristics does not preclude signal diversity within or among populations. The causes of intraspecific call variation have a number of sources, including female preferences and/or pressures to increase environmental transmission clarity in species that occupy a range of habitats. These social and environmental pressures can result in populations of conspecific animals generating calls with slightly different acoustic characteristics.

Geographic or population variation in acoustic communication has been particularly well documented in cricket frogs (*Acris crepitans*). Intraspecific population differences in body size (Nevo, 1973), call characteristics (Nevo & Capranica, 1985; Ryan & Wilczynski, 1991) and auditory tuning (Capranica, Frischkopf & Nevo, 1973; Ryan & Wilczynski, 1988; Keddy-Hector, Wilczynski & Ryan, 1992; Ryan, Perrill & Wilczynski, 1992; Wilczynski, Keddy-Hector & Ryan, 1992) have received special attention. This previous work demonstrated that cricket frog populations show east-west clinal variation in mean body size linked to habitat, with western populations that occupy progressively drier habitats having relatively larger body sizes (Nevo & Capranica, 1985; Ryan & Wilczynski, 1991). In cricket frogs (Nevo & Capranica, 1985; Wagner, 1989; Ryan & Wilczynski, 1991; Keddy-Hector *et al.*, 1992) and other anurans (Wilczynski, Zakon & Brenowitz, 1984; Ryan 1985; Zakon & Wilczynski, 1988) body size correlates strongly with both the dominant frequency of the species-specific call and with the tuning of the peripheral auditory system, specifically the basillar papilla. Larger body size predicts lower-frequency calls and lower-frequency tuning in both call generation and reception systems is probably due to the lower mechanical resonance frequencies typical of larger structures.

The population differences among cricket frogs in body size, therefore, predicts differences in their vocal communication signals and auditory systems. In fact there are such differences in the predicted direction. For example, cricket frog populations differ in call dominant frequency, many temporal call characters, and peripheral auditory tuning, with the western populations having lower frequency characteristics of both systems (Nevo & Capranica, 1985; Ryan & Wilczynski, 1988, 1991; Wilczynski *et al.*, 1992). Originally, a pleiotropic effect of body size was suggested as the cause of population variation in these traits (Nevo & Capranica, 1985).

In previous work (Ryan & Wilczynski, 1991; Keddy-Hector *et al.*, 1992), we have shown that body size differences are not solely responsible for the cricket frog population differences in either call characteristics or tuning. In a recent study of cricket frogs, (McClelland *et al.*, 1996) we found that the morphological structures of the larynx, responsible for call production, and the ear, responsible for call reception, are also positively related to body size and negatively related to the dominant frequency of the call. Because anuran call characteristics have a basis in the morphology of the larynx (Martin, 1971; Martin & Gans, 1972; Schneider, 1988; McClelland & Wilczynski, 1989; Ryan & Drewes, 1990; Wilczynski, McClelland & Rand, 1993; McClelland *et al.*, 1996), it follows that the larynx should show corresponding population variation, and it is of interest to ascertain whether any such population-level differences are independent of body size. In addition, comparing both larynx and ear among populations addresses questions regarding whether the anatomical structures necessary for production and reception in an acoustic communication system are ‘coupled’ and thus would show identical relationships in each population as the system diverges geographically within a species. In this context, we used morphological techniques to examine patterns of population-level variation in the anatomy of the structures used by cricket frogs for acoustic communication.

MATERIAL AND METHODS

Sample selection and call analysis

We examined the laryngeal and ear anatomy of 88 males from eight sites in Texas. Fifty-nine of the cricket frog specimens from six populations had been collected as part of a larger survey and analysis of 17 populations assessing geographic variation in cricket frog call characteristics (see Ryan & Wilczynski, 1991). The eight sites from which the animals were collected are close to a transect at 30.5° (±0.5°) latitude spanning the geographic division between two subspecies (*Acris crepitans crepitans* and *A. c. blanchardi*) and two qualitatively different habitats (i.e. open grassland and forest; Fig. 1). The populations were chosen to include samples from the extreme eastern and western points investigated in our previous study, the transition zone between eastern and western habitats and subspecies, an isolated pine forest habitat in central Texas, and at least two populations within each habitat type.

In our previous study, cricket frog populations along this transect showed a significant level of variation in several characteristics of the field-recorded vocalizations even after correcting for ambient temperature (see Ryan & Wilczynski,

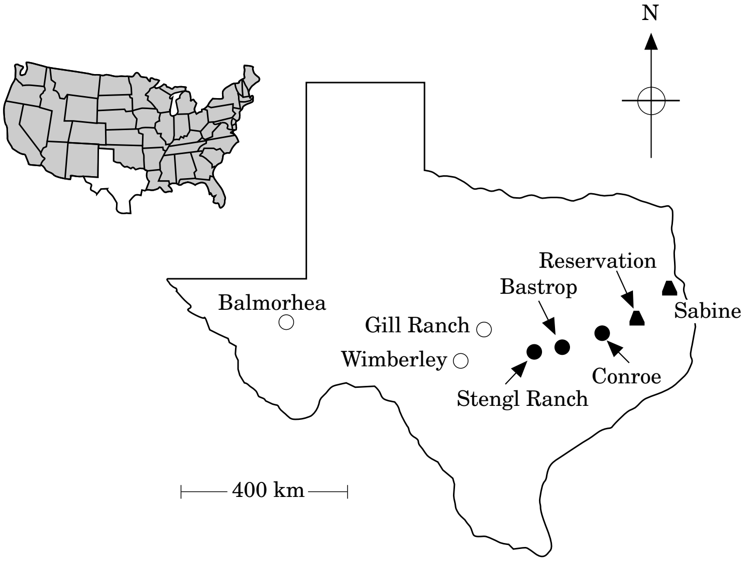

Figure 1. Cricket frogs were collected from eight sites across Texas at approximately 30.5° (±0.5°) latitude. Populations represented two subspecies (*Acris crepitans crepitans* = △, ▲; *A. c. blanchardi* = ○, ●) from open grassland (open symbols) and forested (closed symbols) habitats.

1991 for details regarding call recording and analysis). To determine if there were also significant call differences among this subsample of the populations within the transect, we used data from the six populations included in our previous study to ascertain population level differences in call dominant frequency, call duration, pulse number within the call, and pulse rate (pulse number divided by call duration) using an analysis of variance. Analysis of covariance was also applied to these data to determine if population differences in this subsample remained significant after correction for population differences in body size as in the previous larger sample, and regression analysis was applied to determine if the clinal patterns seen in the earlier study were preserved in this subsample.

Tissue processing and morphological procedures

Calling males from each population were collected and sacrificed by an overdose of the aqueous transdermal anesthesia MS-222 (3-aminobenzoic acid ethyl ester, methanesulfonate salt). 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 with Vernier calipers to the nearest 0.1 mm after fixation.

We processed the specimens so that serial sections showing the ear and larynx could be measured. We began by dissecting the head and throat of each animal, removing as much skin as possible. We placed the heads in a de-calcifying agent (‘Cal-Ex’, Fisher Diagnostics) for 24–36 hours, after which we dehydrated the specimens in ethyl alcohol and xylenes before embedding them in paraffin. We sectioned the heads at a width of 25 μm through the ear and laryngeal region and

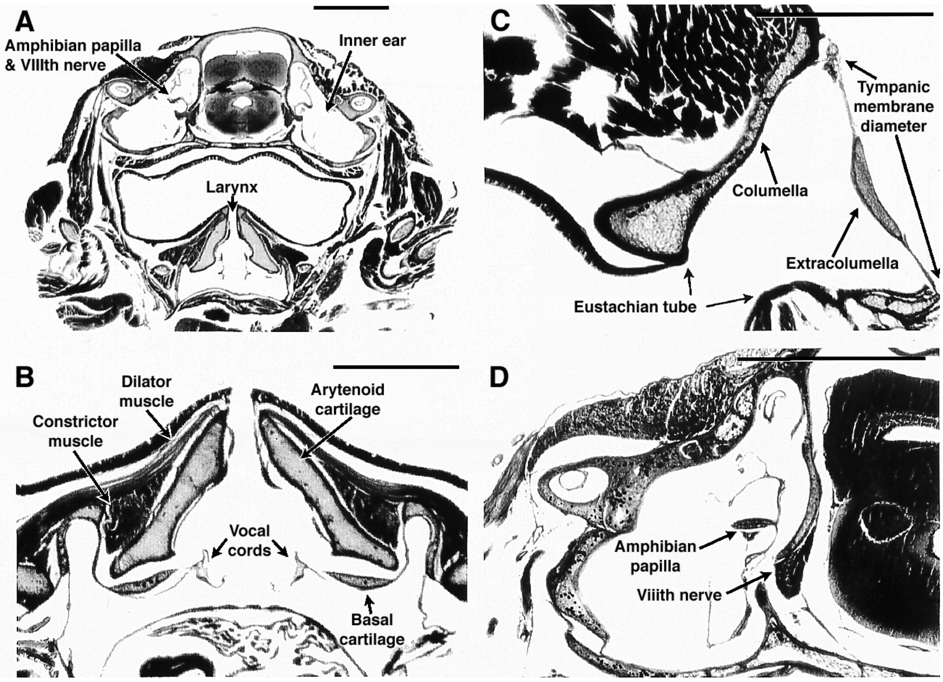

Figure 2. Cross sections (25 μm thick) through the whole head (A), larynx (B), middle (C) and inner ears (D) of a male cricket frog (*Acris crepitans*). Sound is produced by the larynx (A,B) when air is blown across the vocal cords through the glottal slit, setting up a fundamental frequency. Movements 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 tympamic 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 (modified from McClelland *et al.*, 1996).

mounted all tissue slices on subbed slides. We stained the sections with Pollak's trichrome (Humason, 1972) and cresyl violet to differentiate the muscles and cartilage (Fig. 2). We processed the tissues in batches of randomly selected individuals to avoid the potentially confounding problem of inter-batch variation in the effects of dehydration and embedding procedures on morphological measurements.

We measured the laryngeal and ear structures by projecting the slide on a Summagraphic 2201 digitizing pad interfaced with an IBM PC-AT that had been programmed with the 'Sigma-scan' (Version 3.0, Jandel Scientific) graphics software. We traced the perimeter of the relevant areas in every 10th section (i.e. intervals of 250 μm). In the larynx we measured the sizes of arytenoid cartilages, vocal cords, basal cartilage of the arytenoid and the laryngeal constrictor and dilator muscles (see Fig. 2A & B). The measured ear structures included the middle and inner ear cavities, extracolumella cartilages, and tympanic membranes (see Fig. 2C & D). We subsequently calculated the volumes (V_{total}) of each anatomical structure from the area measurements of each slice using the formula:

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

where i is a running index of the order of the measured slices, $\mathrm{i} = 1$ is the first cross-section 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, $A_{\mathrm{i}+1}$ 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 cone frustum (V_{slice}) does not rely on the assumption that the area measurements for each slice are equal to accurately calculate the volume. We also measured the diameter of the tympanic membrane with a linear setting of the 'Sigma-scan' program. Whenever possible, we made bilateral measurements and averaged the resulting volumes or diameters to yield one value for each structure per animal.

Statistical analyses

We calculated means and standard deviations for the morphological measurements for each population and then assessed the significance of the differences among populations with a Tukey's post-hoc multiple comparison test. To determine if body size differences among populations could account for geographic variation in the larynges and ears we first tested the interaction term for significance in each analysis, then we assessed populations differences with an analysis of covariance (ANCOVA) using snout-vent length (SVL) as the covariate.

In order to assess geographic patterns of variation, specifically whether the populations show a clinal trend in the size of their anatomical traits, we used population mean as the dependent variable (from Table 1), and examined the Pearson product-moment correlations of morphological trait size and the longitude of the site. We also examined patterns of clinal variation in the characteristics independent of body size by determining the residuals from the regression of trait on SVL, then correlating the mean residual score for each population with longitude. In this way, we could determine whether there were clinal trends not only in the raw scores of the traits, but also in the sizes of the traits relative to SVL.

RESULTS

Call characteristics

The analysis of calls from six of the investigated sites confirms that significant population variation, as previously seen in our analysis of 17 populations (Ryan & Wilczynski, 1991), can also be observed in this subset of the larger sample (Tukey's post-hoc multiple comparison test, all $P < 0.01$; Table 1). As reported in Ryan and Wilczynski (1991) Balmorhea frogs, at the western edge of the sampled range, have the lowest call dominant frequency, slowest pulse rate, least number of pulses, and the longest calls of the six populations included in this study. Samples from Conroe show the second-lowest call dominant frequency, but the pulse rates at this site are the highest of all the measured populations. Cricket frogs from Bastrop and Conroe, in the middle of the sampled range, have the shortest calls and Gill ranch frogs show the most number of pulses in their calls. As previously reported, call characteristics are different among populations even when the variation due to body size is eliminated with an analysis of covariance (Table 2). Variation in dominant frequency among the populations is clinal, confirming that higher call frequencies are found in the easternmost populations and decrease in a westerly direction (Table 3). In the populations we examined, temporal call parameters do not show a clinal pattern and after correction for body size, none of the call parameters, including dominant frequency, show clinal variation.

Morphological characteristics

All the morphological characteristics examined in this study show significant population variation among the eight sites (Tukey's post-hoc multiple comparison test; Table 1). Animals from Balmorhea have the largest body size, followed by Conroe and Bastrop (Fig. 3A). Wimberley and Stengl sites produced the smallest males. Laryngeal size differences follow the same trend as the body size (Fig. 3). Ear components, although showing significant population variation, do not vary in the same way as the larynx (Fig. 4). For instance, mean tympanic membrane diameter is largest in Conroe but this site, although having the second largest mean body and larynx sizes, has only the fourth largest mean extracolumellar volume (Table 1; Fig. 4B).

Results of the univariate analysis of covariance (ANCOVA) indicate that most anatomical characteristics are significantly different among populations even after the effects of body size differences are statistically removed (Table 2). Vocal cord volume in the larynx and extracolumella volume in the ear are the exceptions to this general finding. In the raw-score analysis, none of the morphological traits showed clinal variation across the sampled range (Table 3). When differences in the morphological characteristics due to body size variation are eliminated, head width and three laryngeal traits, arytenoid cartilage, vocal cord, and dilator muscle volumes, do show a clinal trend. Furthermore, the direction of the trends (positive) indicates that the western populations have larger laryngeal structures than would be predicted from the species-wide correlations of traits with body size (Table 3; Fig. 4A & B). However, none of the ear traits show a similar trend across the geographic range sampled in this study (Table 3).

DISCUSSION

Call variation

Geographic variation in call characteristics and morphology is a well-documented phenomenon in cricket frogs. Call dominant frequency as well as some temporal characteristics show differences among both widely separated populations (Nevo & Capranica, 1985; Ryan *et al.*, 1992) and geographically proximate areas (Ryan &

Table 1. Means (±SD) of body, laryngeal and ear measurements of male cricket frogs (*Acris crepitans*) from eight populations. Sample sizes (*n*) and longitude (in degrees) are shown below the population abbreviations

	Balm (10) 103.66	Wimb (11) 98.00	Gill (16) 97.85	Sten (12) 97.30	Bast (9) 97.25	Conr (10) 95.50	Resv (10) 95.00	Sabn (10) 93.90
Cell characteristics								
Dominant frequency (kHz)	2.710 (±0.254)	N/A	3.557 (±0.152)	N/A	3.764 (±0.115)	3.524 (±0.106)	3.847 (±0.177)	3.987** (±0.108)
Call duration (ms)	35.475 (5.952)	N/A	32.030 (±8.724)	N/A	25.157 (±4.886)	25.421 (±7.379)	27.059 (±5.035)	30.958** (±6.900)
Number of pulses in call	4.032 (±0.552)	N/A	6.232 (±1.420)	N/A	4.891 (±0.873)	5.380 (±1.225)	4.680 (±1.133)	4.658** (±0.682)
Pulse rate (pulses/s)	115.402 (±17.770)	N/A	197.794 (±21.933)	N/A	195.882 (±22.029)	215.912 (±26.947)	173.963 (±34.708)	154.786** (±30.095)
Body and head size								
Snout-vent length (mm)	28.62 (±0.77)	20.42 (±2.06)	22.89 (±0.90)	20.80 (±1.60)	24.49 (±1.24)	24.50 (±0.86)	22.90 (±0.88)	23.43** (±0.89)
Head width (mm)	9.08 (±0.36)	6.67 (±0.73)	7.60 (±0.41)	6.71 (±0.70)	8.21 (±0.34)	8.51 (±0.31)	7.91 (±0.26)	7.91** (±0.20)
Laryngeal volume measurements								
Arytenoid cartilage volume (mm^3)	0.867 (±0.090)	0.362 (±0.135)	0.582 (±0.086)	0.478 (±0.148)	0.622 (±0.111)	0.812 (±0.131)	0.654 (±0.096)	0.661** (±0.112)
Vocal cord volume (mm^3)	0.065 (±0.006)	0.031 (±0.012)	0.041 (±0.009)	0.035 (±0.008)	0.047 (±0.007)	0.057 (±0.008)	0.047 (±0.007)	0.048** (±0.010)
Basal cartilage volume (mm^3)	0.036 (±0.008)	0.015 (±0.009)	0.028 (±0.007)	0.020 (±0.008)	0.023 (±0.006)	0.030 (±0.004)	0.027 (±0.007)	0.026** (±0.004)
Constrictor muscle volume (mm^3)	0.778 (±0.115)	0.253 (±0.117)	0.456 (±0.132)	0.329 (±0.132)	0.481 (±0.058)	0.520 (±0.076	0.510 (±0.089)	0.501** (±0.072)
Dilator muscle volume (mm^3)	0.265 (±0.032)	0.092 (±0.052)	0.163 (±0.063)	0.124 (±0.061)	0.172 (±0.022)	0.245 (±0.091)	0.181 (±0.041)	0.174** (±0.026)

TABLE 1. contd.

	Balm (10) 103.66	Wimb (11) 98.00	Gill (16) 97.85	Sten (12) 97.30	Bast (9) 97.25	Conr (10) 95.50	Resv (10) 95.00	Sabn (10) 93.90
Ear volume and diameter measurements								
Tympanic membrane diameter (mm)	1.018	0.899	1.083	1.041	1.052	1.158	1.050	0.975**
	(±0.064)	(±0.117)	(±0.098)	(±0.100)	(±0.097)	(±0.106)	(±0.062)	(±0.055)
Extra columella volume (mm^3)	0.027	0.009	0.016	0.016	0.022	0.020	0.020	0.020**
	(±0.005)	(±0.007)	(±0.008)	(±0.004)	(±0.004)	(±0.004)	(±0.008)	(±0.007)
Middle ear volume (mm^3)	0.452	0.170	0.239	0.202	0.247	0.320	0.197	0.271**
	(±0.086)	(±0.061)	(±0.077)	(±0.057)	(±0.061)	(±0.040)	(±0.072)	(±0.046)
Inner ear volume (mm^6)	1.435	0.937	1.112	1.040	1.126	1.399	1.029	1.141**
	(±0.208)	(±0.213)	(±0.246)	(±0.227)	(±0.152)	(±0.192)	(±0.235)	(±0.125)

Tukey's post-hoc multiple comparison test for eight populations.
*$P<0.05$, **$P<0.01$.

Table 2. Results of the analysis of covariance (*F*-ratios) among cricket frogs with SVL as the covariate. Call characteristics are from six Texas populations (after Ryan & Wilczynski, 1991) and the morphological characteristics are from eight populations

	Snout-vent length
Cell characteristics	
Dominant frequency	22.642**
Call duration	2.702*
Number of pulses in calls	2.849*
Pulse rate	10.898**
Morphological measurements	
Head width	5.466**
Larynx	
Arytenoid cartilage volume	5.435**
Vocal cord volume	1.687
Basal cartilage volume	2.845*
Constrictor muscle volume	2.508*
Dilator muscle volume	2.626*
Ear	
Tympanic membrane diameter	9.614*
Extracolumella volume	1.005
Middle ear volume	3.417**
Inner ear volume	2.820*

Results of ANCOVA: *$P<.05$, **$P<.01$.

Table 3. Results of the correlation (Pearson's product-moment correlation, *r*) of characteristics with longitude using population means of the raw scores (Tables 1 & 2) and residuals from the regression of trait size on snout-vent length

Characteristics	Raw scores	Residuals
Vocalization characteristics ($n=6$)		
Dominant frequency	−0.943**	−0.545
Call duration	0.659	0.294
Number of pulses in call	−0.326	0.205
Call pulse rate	−0.584	0.123
Body and head size ($n=8$)		
Snout-vent length	0.564	—
Head width	0.309	0.926**
Laryngeal measurements ($n=8$)		
Arytenoid cartilage	0.237	0.864**
Vocal cord	0.343	0.821*
Basal cartilage	0.360	0.634
Constrictor muscle	0.473	0.669
Dilator muscle	0.315	0.748*
Ear measurements ($n=8$)		
Tympanic membrane	−0.146	0.627
Extra columella	0.318	0.648
Middle ear	0.615	0.317
Inner ear	0.412	0.594

*$P<.05$, **$P<.01$.

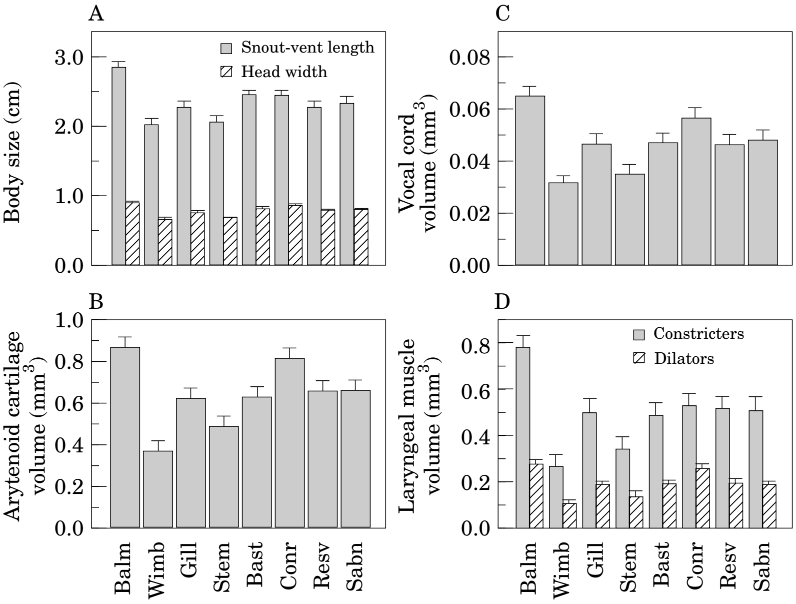

Figure 3. Population means of cricket frog snout-vent length and head width (A) and anatomical components of the larynx (B,C,D) from eight sites in Texas arranged from west to east. Laryngeal measurements include the volumes of arytenoid cartilages (B), vocal cords (C) and the constrictor and dilator muscles (D). The populations differ significantly in all morphological characteristics (Tukey's post-hoc multiple comparison tests, $P<.01$). (Error bars = SEM; populations abbreviations: Balm = Balmorhea, Wimb = Wimberlay, Gill = Gill ranch, Sten = Stengl ranch, Bast = Bastrop, Conr = Conroe, Resv = Reservation, Sabn = Sabine).

Wilczynski, 1988, 1991). Our analysis of a subset of the call data from a previous study (see Ryan & Wilczynski, 1991), indicates that our sample populations have significant differences in call dominant frequency, call duration, number of pulses in the calls and pulse rate (Table 1). Furthermore, as in that study's analysis of the larger sample, the significant call differences remain after correction for body size differences among populations.

Our subsample does not completely preserve the geographic patterns apparent in a wider sampling along this transect. The clinal trend in dominant frequency from high frequency in the east to low frequency in the west is apparent, but clinal trends in call duration (short to long in eastern to western populations) are not seen. This is not a serious problem for this study, because our goal is to examine population variation in the morphology of the structures that are responsible for the generation and reception of vocal communication signals. Given that our call analysis indicates significant spectral and temporal call variation among populations both before and after correction for body size differences, the populations we selected provide an appropriate substrate for examining this relationship.

Larynx morphology

Correlations between call characteristics and the size of the larynx have a basis in the biomechanics and physiology of sound production (McClelland *et al.*, 1996),

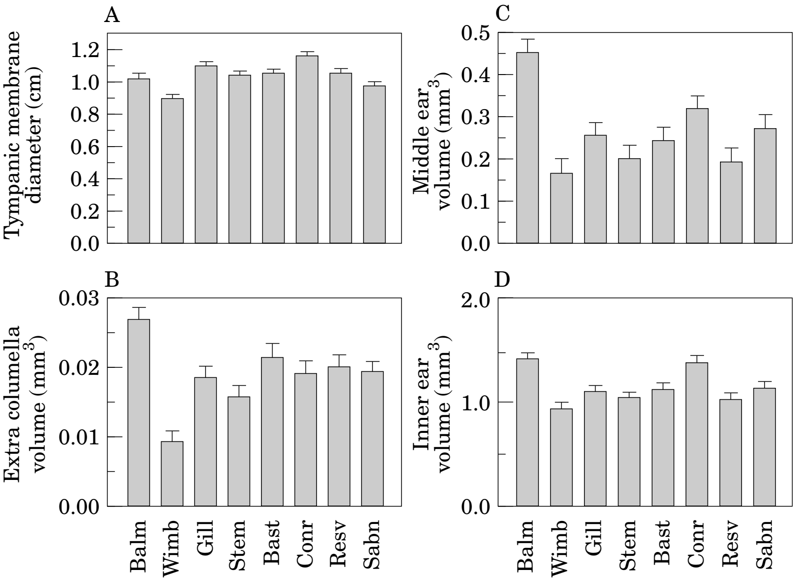

Figure 4. Population means of male cricket frog ear components including the tympanic membrane diameter (A) and volumes of the extracolumella cartilage (B), middle (C) and inner (D) ear cavities from eight Texas populations arranged from west to east (left-right). The populations differ significantly in all measured ear traits (Tukey's post-hoc multiple comparison tests, $P<.01$). (Error bars = SEM; see Fig 1. for population abbreviations.)

and because call characteristics vary among cricket frog populations, we predicted that the laryngeal morphology would also show differences. This prediction was confirmed as all the laryngeal components demonstrate a significant level of variation among populations (Table 1).

Within this species, body size is a significant predictor of laryngeal and ear size (McClelland *et al.*, 1996). Larger frogs, in general, have larger larynges, although population differences in the laryngeal characteristics (except vocal cord volume) are also apparent after the effects of body size are removed, as demonstrated by the ANCOVA results. This indicates that body size has a significant influence on the size of laryngeal characters across populations (McClelland *et al.*, 1996), but differences in body size do not completely explain variation in these traits among populations, just as our previous study (Ryan & Wilczynski, 1991) found that differences in body size could not account for all the geographic variation in call characteristics in cricket frogs. This means that cricket frog populations differ not only in absolute body and larynx size, but also in larynx size relative to body size.

It is interesting to note that vocal cord volumes, which presumably have a strong influence on call dominant frequency (Schmid, 1978), are not different among the cricket frog populations after correction for body size (SVL), despite a significant level of geographic variation in dominant frequency independent of body size (Table 2). Although measurement error could obscure such differences, it is also possible that in cricket frogs other features of the call generation system, such as arytenoid or basal cartilages, have an unexpectedly significant influence on dominant frequency (McClelland *et al.*, 1996), which could account for the observed population differences that are greater than those predicted by the mathematical relationship of body size with vocal cord volume.

One interesting aspect of population variation seen in this analysis is that head width, arytenoid cartilage, vocal cord and dilator muscle volume show clinal trends across the range after correction for body size. In each case, the analysis indicates that progressively more western populations have correspondingly larger larynges than their body size would predict. Generalizing this finding to the larger sample reported previously (Ryan & Wilczynski, 1991) would explain the clinal variation in call dominant frequency, which decreases from east to west in this species. In our subsample, we saw such a clinal effect in the uncorrected scores for dominant frequency. The correlation with longitude in the corrected scores, although strongly positive, was not statistically significant, perhaps due to the restricted number of populations included.

The partial uncoupling of laryngeal component from body size in cricket frogs has implications for the evolutionary response of these animals to selection. It suggests that, within constraints, body size and larynges are able to differentially respond to types or degrees of selection. Selection operating on call characteristics, such as those to increase transmission clarity or attractiveness to females (Ryan *et al.*, 1990, 1992), might be operating directly via changes in the laryngeal morphology as well as indirectly via body size. Conversely, it also might be possible for selection associated with environmental differences in temperature or humidity (Nevo, 1973; Nevo & Capranica, 1985) to operate on body size while conserving laryngeal structure and thus also the species typical call parameters. This results in differences in the size relationships of the larynx to body size among populations, and concurrently, differences in the relationships of call characteristics and body size.

Ear morphology

The components of cricket frog ears measured in this study all show significant size differences among populations (see Fig. 4, Table 1). As all measured ear structures, except tympanic membrane diameter, correlate with body size in cricket frogs (McClelland *et al.*, 1996), population differences in body size no doubt contribute to this. With the exception of extracolumella volume, the ear components also differed among populations after the statistical correction for body size (Table 2). This indicates that, similar to the situation with calls and larynges, changes in body size across the range contribute to, but do not fully explain, population differences in the sizes of ear components.

Geographic differences in ear structure would be predicted based on the correlations of ear component sizes with dominant frequency shown on a species-wide basis in a previous report (McClelland *et al.*, 1996). Those correlations suggest that changes in ear components might reflect a mechanism to enhance auditory sensitivity to certain spectral parameters associated with a population's call or habitat. In mammals, cochlea size among gerbil species differs depending on the frequency to which they are tuned, and this corresponds to the acoustic environments encountered by the species. Gerbils occupying dry habitats have larger tympanic membranes, bulla and stapes footplates, possibly as an adaptation to enhance the reception of low frequency sounds produced by conspecific foot-drumming or abiotic desert sounds (Plassmann, Peetz & Schmidt, 1987). Similarly, in cricket frogs, changes in ear size might contribute to population differences in the tuning of the peripheral auditory system (Capranica *et al.*, 1973; Keddy-Hector *et al.*, 1992; Wilczynski *et al.*, 1992) that maintain a rough match with the population's call frequency.

There is a general tendency for ear traits to follow the same population size trends as larynx structures. This may be due largely to the correlation of both systems with body size (McClelland *et al.*, 1996). However, despite the necessity to coordinate the sending and receiving portions of an acoustic communication system, the volumes of ear structures do not vary among populations in the same way as the laryngeal characteristics responsible for generating vocalizations. For example, extracolumella size is smaller in Conroe males than in males from more eastern populations even though Conroe laryngeal cartilages and vocal cords are considerably larger. Tympanic membrane diameter does not vary among populations in the same way as laryngeal traits. Furthermore, a clinal component to the relationship of larynx size to body size progressively and proportionally increases larynx size in more western populations. Ear traits, however, do not show this clinal trend either in raw scores or in the size of the components relative to body size (Table 3). This implies that, although the larynx and ear might have functionally coevolved to be generally similar in size, the peripheral anatomy of these structures are at least partly uncoupled. To some extent, auditory systems are adapted to preferentially process sounds of specific, biologically relevant acoustic characteristics, but they are also capable of receiving input from a much wider variety of sources, both biotic and abiotic. Thus, ears fulfill a more general function than larynges. Perhaps this incorporates a significant amount of flexibility into the auditory system and removes peripheral ear size from the necessity to evolve in strict correlation with laryngeal sizes or specific characteristics of the advertisement vocalizations. Regardless of the reason that ear and laryngeal structures show population differences in their relative sizes, the patterns of variation in ear and larynx volumes supports the concept of differential responses to selection pressures by these two aspects of the communication system.

Middle and inner ear cavities account for a significant portion of the head width. Based on the relationship between ears and head size, one would expect ear measurements to show the same pattern of geographic variation as head width. In cricket frogs, head size, but not ear characteristics, shows clinal variation among the populations (Table 3), with the head becoming progressively larger relative to body size in more western populations. Thus, in this species, changes in head size either linked to overall changes in body size or independent of it, are probably not the only agents of change resulting in different ear sizes across the species range.

Mechanisms of variation

Previous work in cricket frogs has highlighted a complicated set of selection pressures contributing to call and auditory tuning diversity within this species (Ryan & Wilczynski, 1988, 1991). These forces must serve as the ultimate causes of laryngeal and ear diversity also, because these structures are the substrate of the behavior and physiology. In the eastern part of the investigated cricket frog range, environmental constraints of the forest habitat require acoustic call parameters exhibiting fast pulse rates and high frequency calls to increase transmission clarity (Ryan *et al.*, 1990). These call parameters are generally correlated with smaller larynges (McClelland *et* *al.*, 1996). Conversely, the more open habitats show little effect on call degradation (Ryan *et al.*, 1990), but their aridity exerts a different environmental pressure, resulting in frogs of larger bodies (Nevo, 1973) with concomitantly larger larynges. Furthermore, sexual selection in the form of a female preference for male calls of lower frequency (Ryan *et al.*, 1992) is probably a constant force across the entire species range, and, unopposed in the western populations by habitat selection, might exaggerate the low frequency nature of the call and the relative size of the larynx. The clinal nature of these differences across the range follows logically from the observation that Texas herpetofauna biomes are not discrete, but change gradually in an east-west direction that may allow populations to come into contact (Owen & Dixon, 1989).

The proximal mechanism for generating the population differences cannot be addressed by this study. However, it has been suggested that population differences in basilar papilla tuning and call characteristics represent differences in the relationship of specific morphological traits with body size (Keddy-Hector *et al.*, 1992) that might stem from developmental differences among populations. This is confirmed in the current study by the clinal trends seen in the residuals of the regression of trait on body size (Table 3). Initial growth phase differences or different starting points in the ontological growth rates of the resonant structures in the ear or larynx have been proposed as possible mechanisms by which population variation could result (Keddy-Hector *et al.*, 1992). An examination of the developmental processes, including hormonal influences that play a large role in male laryngeal development (Kelley, 1986), would provide evidence to confirm or disprove the proposed mechanisms.

General conclusions

In previous studies, we (Ryan & Wilczynski, 1991; Wilczynski *et al.*, 1992) showed significant population-level variation in the body size, calls and auditory tuning of cricket frogs. The present paper extends that work by showing significant population variation in the size of the body, head, larynx, and ear in this species. The larynx and ear differences suggest the mechanisms by which the behavioral and physiological population difference are generated. Changes in the sizes of the components of these structures can modify their resonant properties and the muscular activity controlling their movement (McClelland & Wilczynski, 1989; Wilczynski, McClelland & Rand, 1993; McClelland *et al.*, 1996), thus producing call and tuning differences distinguishing the communication systems of each population.

Despite positive correlations among body, larynx, and ear sizes (McClelland *et al.*, 1996), these three morphological systems demonstrate a significant degree of independence in their development among populations. This suggests that, within a species, each can respond differently to selection pressures and other evolutionary events to some degree, thereby leading to a unique matrix of body, larynx and ear characteristics defining each population and matching these traits to a particular habitat.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of Howard M. Liljestrand. We declare that the experiments complied with the Principles of Animal Care, publication No.

86-23, revised 1985, of the National Institute of Health and also with the current laws of the United States of America. This research was supported by NSF BNS 9021185 and NIMH RO1 MH45696.

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