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Evolutionary and Allometric Insights into Anuran Auditory Sensitivity and Morphology

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Abstract

As species change through evolutionary time, the neurological and morphological structures that underlie behavioral systems typically remain coordinated. This is especially important for communication systems, in which these structures must remain coordinated both within and between senders and receivers for successful information transfer. The acoustic communication of anurans ("frogs") offers an excellent system to ask when and how such coordination is maintained, and to allow researchers to dissociate allometric effects from independent correlated evolution. Anurans constitute one of the most speciose groups of vocalizing vertebrates, and females typically rely on vocalizations to localize males for reproduction. Here, we compile and compare data on various aspects of auditory morphology, hearing sensitivity, and call-dominant frequency across 81 species of anurans. We find robust, phylogenetically independent scaling effects of body size for all features measured. Furthermore, after accounting for body size, we find preliminary evidence that morphological evolution beyond allometry can correlate with hearing sensitivity and dominant frequency. These data provide foundational results regarding constraints imposed by body size on communication systems and motivate further data collection and analysis using comparative approaches across the numerous anuran species.

Introduction

Most behaviors require complementarity among a variety of phenotypic traits for optimal performance. For these behaviors to remain functional as new species arise over evolutionary time, there must be coordinated evolution of these traits. Acoustic communication is a good example of the importance of maintaining functional coordination. Communication could fail if changes to hearing in receivers push auditory sensitivity outside the range of the species' acoustic signal, or if signal properties shift in response to environmental noise, for example, without a concomitant change in hearing range.

Allometry, the scaling relationships between body size and aspects of morphology, physiology, and behavior, is one mechanism that could facilitate coordination of communication systems and has long been of interest to biologists [Huxley, 1932; Gould, 1977; Schmidt-Nielsen, 1984; Gillooly and Ophir, 2010; Tonini et al., 2020]. Thus,

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similarities in morphology of related species arising from correlated changes with body size could contribute to maintaining functional correlations of traits within a specific domain. Therefore, such functional correlations that appear to evolve together may in fact emerge as a byproduct of changes to body size. To understand phenotypic evolution, it is necessary to account for both differences in body size as well as evolutionary relationships amongst taxa being compared. Indeed, there is a large body of research comparing brain and body size evolution that explores what factors can account for deviations in allometric scaling between the brain and body size across taxa [Eberhard and Wcislo, 2011; Corfield et al., 2016; Montgomery et al., 2016; Mai and Liao, 2019].

This dissociation between evolutionary relationships and body size comparisons is especially important in acoustic communication systems. On the vocal production side, there tends to be a negative relationship across species between body size and spectral components of the vocalization, especially the dominant frequency [Wallschläger, 1980; Fletcher, 2004; Gingras et al., 2013; Bowling et al., 2017]. As body size increases so does the size of the vocalization mechanisms that contribute to the spectral parameters of the vocalization [Goller and Riede, 2013; Riede and Goller, 2014]. This scaling of body size and call acoustic properties appears quite strong, with only a few cases in which anuran clades have "escaped" this allometric constraint [Tonini et al., 2020].

On the acoustic perception side, there are fewer studies relating hearing sensitivity or frequency range to body size [Loftus-Hills, 1973; Wilczynski and Capranica, 1984; Wilczynski et al., 1984]. When hearing sensitivity improves with increased body size, it is assumed to be due to related size increases of auditory morphology. Specifically, the interaural distance correlates with high-frequency hearing from the mouse to the elephant [Heffner and Heffner, 1980]. This relationship, however, need not be immutable; this same relationship was not observed in dogs from the Chihuahua to the St Bernard [Heffner, 1983]. Correlations between body size and hearing sensitivity could also arise from body size correlates with other aspects of auditory morphology. For example, middle ear performance varies with body size from sand cats to tigers [Huang et al., 2000].

Many of these cross-species comparisons in acoustic communication neglect to control for phylogenetic relatedness among species, which is crucial since we cannot assume that species are independent samples [Felsenstein, 1985; Freckleton et al., 2002]. In addition, such studies typically compare body size to hearing sensitivity or to vocalizations but rarely consider all three parameters, let alone the morphological characters that underlie hearing ability.

One study that addresses all three variables with proper phylogenetic controls analyzed the low-frequency isolation calls by pups in a number of bat species [Bohn et al., 2006]. They asked whether low-frequency hearing in bats exhibits correlated evolution with (i) body size, (ii) high-frequency hearing sensitivity, (iii) high echolocation call frequency, and (iv) low pup isolation call frequency. They found that low-frequency hearing exhibits correlated evolution with high-frequency hearing and echolocation call frequency, but not with body size. Once high-frequency hearing was accounted for, then a relationship with pup isolation call frequency emerged, suggesting correlated evolution between low-frequency sensitivity and pup isolation call frequency. This study shows the complexities that can arise when accounting for variation in acoustic communication systems across phylogenies.

Anurans ("frogs") offer an excellent opportunity for such studies comparing aspects of acoustic communication systems while accounting for both body size and evolutionary history. Thousands of frog species are known to engage in acoustic communication, providing ample breadth to ask questions regarding evolutionary changes to acoustic production and perception [Wells and Schwartz, 2006; Chen and Wiens, 2020]. Moreover, frogs exhibit large variation in body size [Womack and Bell, 2020]. For example, the largest frog in this study (Colorado river toad; *Incilius alvarius*) is more than 7 times longer than the smallest frog in this study (eastern sign-bearing froglet; *Crinia parinsignifera*), allowing us to ask questions regarding how body size correlates with aspects of acoustic communication.

The primary pathway for sound perception in frogs involves sound waves first impinging on tympanic membranes (or tympana), which are situated externally on the side of the frog's head just posterior to the eyes in most species [Mason, 2006]. (Interestingly, the tympanum may also help transmit calls from males [Purgue, 1997]). From there, sound is transmitted through the middle ear to the inner ear where it is perceived by two distinct sensory organs: the basilar papilla and the amphibian papilla [Smotherman and Narins, 2000; Simmons et al., 2006]. Hair cells within each organ are responsible for detecting sound, and the amphibian papilla contains more hair cells which are tuned to lower frequencies compared to the hair cells in the basilar papilla [Wilczynski and Capranica, 1984; Simmons et al., 2006]. Thus, frogs typically have two peaks in hearing sensitivity at both a low and a high frequency. The tuning of the basilar papilla is always higher in frequency than the amphibian papilla, and often corresponds quite closely to the dominant frequency of the males' courtship call [Gerhardt and Schwartz, 2001]. Indeed, species with both low and high emphasized frequencies in their calls tend to have tuning in each papilla that matches each emphasized frequency [Frishkopf et al., 1968; Gerhardt and Schwartz, 2001; Richards, 2006].

Here, we compare previously published datasets to address whether the frog acoustic communication system changes primarily as a byproduct of changes to body size, or whether aspects could evolve together independently from body size. For this, we compared body size to important components of frog auditory morphology (tympanum area [TA] and hair cell counts) and measures of sound sensitivity as well as an aspect of acoustic production (call dominant frequency). We make all these comparisons controlling for phylogenetic relationships.

Materials and Methods

Data Curation

This study is composed of analyses integrating previously published datasets. We were interested in four measures of morphology, which included snout-vent length (SVL) and three measures specific to the auditory system of frogs: TA, amphibian papilla hair cell count (APH) and basilar papilla hair cell count (BPH). Our aim was to compare such morphological measures to auditory sensitivity as well as mating call dominant frequency (DF). Figure 1a depicts a schematic of these features and their relationships to each other.

We used the following sources:

Fox [1995]: A table compiling data for SVL, TA, APH and BPH cell counts, based primarily on Wever [1985].

Taylor et al. [2019]: A table summarizing studies of frog auditory sensitivity using a variety of methods.

Gingras et al. [2013]: A dataset that included SVL and DF.

Tonini et al. [2020]: A dataset that included SVL and DF.

Penna et al. [1990]: Three additional TAs.

To best align morphological measures with body size, we used SVL from Fox [1995] when available. For other species, we averaged the SVL from the other sources, and we also averaged dominant frequency when available from multiple sources.

Overall, we obtained at least one measure of auditory morphology or auditory sensitivity for 81 species (Fig. 1b). Of these 81 species, we found SVL for all 81 species, APH for 48 species, BPH for 42 species, TA for 58 species, auditory sensitivity for 34 species, and DF for 66 species. Unfortunately, we found that most data had very little overlap in the same set of species (e.g., only 9 species had both a BPH and auditory sensitivity measurement). This uneven sampling across species prevented us from running large models with many different predictors. Instead, we focus on the fact that these measures are generally predicted to correlate with body size [Fox, 1995; Gingras et al., 2013; Tonini et al., 2020], which we confirm with phylogenetically controlled analyses. We then ask whether pairs of traits could relate to one another independent of their shared correlation with body size.

We based our measurements of hearing sensitivity on a literature review summarized in Taylor et al. [2019], which covers measurements using a variety of methods. Because frogs generally have sensitivity peaks in both a low frequency and high-frequency range (which typically corresponds to the AP and BP sensitivity peak, respectively), we examined all references in Taylor et al. [2019] and differentiated between these two peaks [Frishkopf and Goldstein, 1963; Loftus-Hills and Johnstone, 1970; Lombard and Straughan, 1974; Capranica and Moffat, 1975; Brzoska et al., 1977; Hubl and Schneider, 1979; Walkowiak, 1980; Fuzessery and Feng, 1982; Hillery, 1984; Wilczynski et al., 1984, 1992; Megela-Simmons et al., 1985; Zelick and Narins, 1985; Penna et al., 1990, 1992, 2008, 2013, 2015; McClelland et al., 1997; Bosch and Wilczynski, 2003; Beckers and Schul, 2004; Katbamna et al., 2006; Yu et al., 2006; Bee and Schwartz, 2009; Miranda and Wilczynski, 2009b; Vélez et al., 2012; Zhang et al., 2012; Buerkle et al., 2014; Schrode et al., 2014; Gall and Wilczynski, 2015; Lee et al., 2017]. See Taylor et al. [2019] for details on the species and methods used in each study.

Across studies of hearing sensitivity, most methods used electrophysiological techniques in which immobilized frogs were exposed to sound playback of varying amplitude, and action potential responses were measured using electrodes. In this study, we used the midpoint of the range of lowest thresholds observed across individuals when available. Electrophysiological measurements were taken from the torus semicircularis (a midbrain auditory structure, using multi- or single unit electrodes) or the 8th nerve (using single unit electrodes). Neurological thresholds have also been measured using evoked potentials recorded from the auditory brainstem response (ABR). Behavioral measures of auditory thresholds include phonotaxis (females) and evoked calling (males) in which the minimum amplitude of playback to elicit a response is measured. These behavioral techniques typically use a species-typical acoustic call as the stimulus, and thus do not differentiate low and high-frequency sensitivity peaks. Rarer techniques include a reflex modification procedure, in which acoustic playback prior to a mild electrical shock can inhibit a frog's reflexive hind leg flexion [Megela-Simmons et al., 1985], as well as a galvanic skin response which measures evoked potential in the skin [electrodermal response; Brzoska et al., 1977]. Because these rarer techniques have only been used in one or two species, and because the sensitivities were not similar to other techniques, we did not include these in our analyses. We rarely had sex-specific information for auditory sensitivity, and thus we averaged across sex.

For visualization, we color-coded data points throughout the results by categorizing species into taxonomic groups within Anura (Fig. 1b). At the broadest level, we categorized species into the suborders Archaeobatrachia, Mesobatrachia, and Neobatrachia. Within Neobatrachia, we additionally grouped frogs into the three families with the largest number of species in our dataset: Bufonidae ("true toads"), Ranidae ("true frogs") and Hylidae ("tree frogs and their allies"). While sometimes considered to be distinct families, we included Pelodryadidae and Phyllomedusidae as part of Hylidae and Pyxicephalidae as part of Ranidae. No other family in Neobatrachia was represented by more than a few species in our dataset; therefore, these families were not individually identified and instead were all color-coded the same.

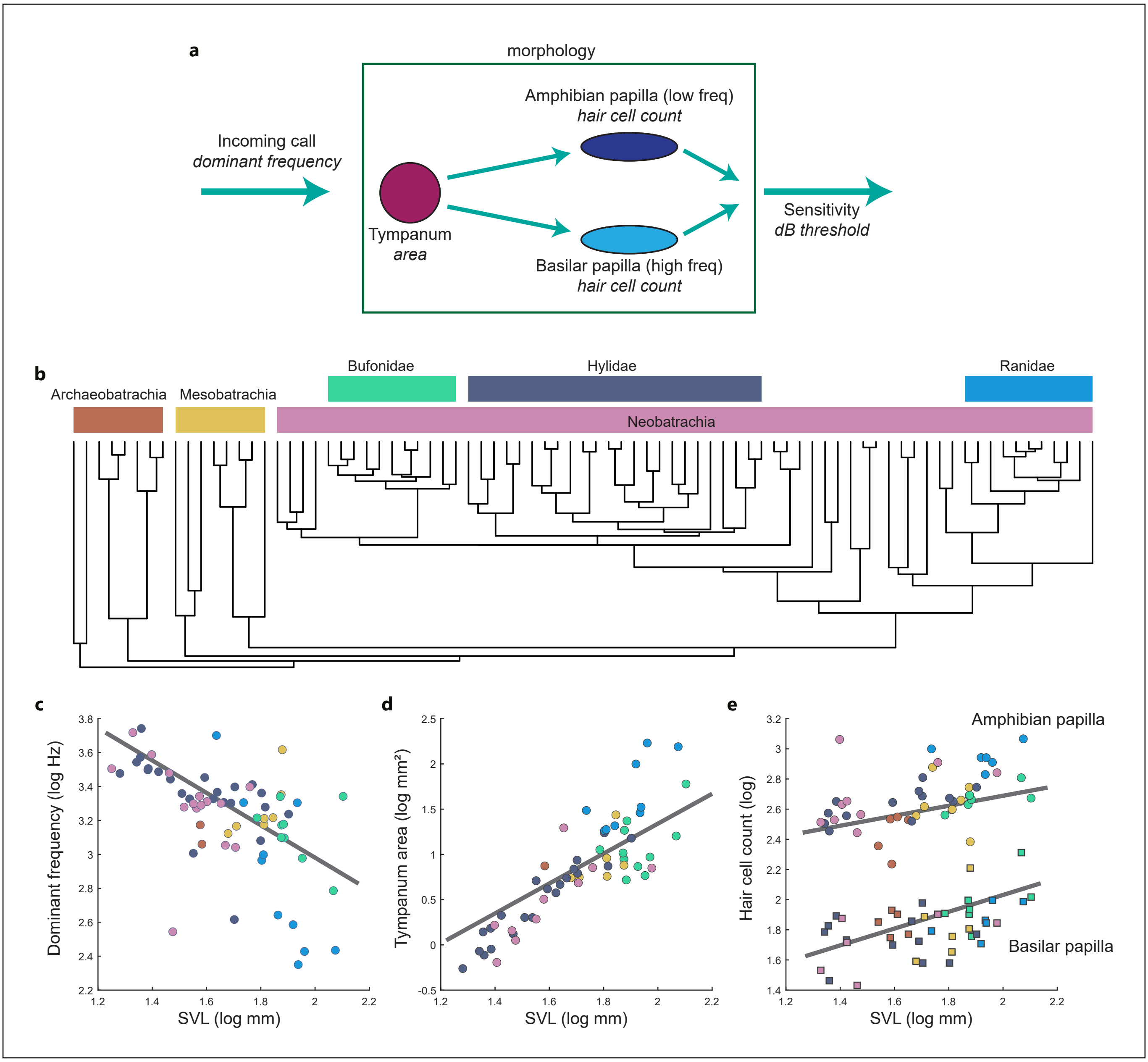

Fig. 1. Auditory system measurements correlate with body size. **a** A schematic showing the various features of interest, with the measurements we analyzed in italics. **b** One phylogenetic hypothesis that depicts all species that were used in at least one analysis. **c–e** Correlations between auditory measurements and body size. We found significant correlations between snout-vent length (SVL) and dominant frequency (**b**), tympanum area (**c**), amphibian papilla hair cell count (**d**; circles) and basilar papilla hair cell count (**d**; squares). All trend lines depict the output of phylogenetically controlled correlations, and all points are color coded to match the bars above the phylogeny in **b**.

Statistical Analyses

Our primary results use each species as a data point and employ phylogenetic generalized least squares (PGLS) models to account for the shared phylogenetic histories among species. All data were log-transformed except for auditory sensitivity (which was measured in decibels, and thus, already is a log scale).

We conducted PGLS correlations in R using the *pgls* function in the “caper’ package [Orme et al., 2012]. All analyses were done using the default parameter settings (lambda, kappa, and delta set at 1). Every PGLS correlation was done on 1,000 trees randomly sampled from the posterior distribution of the AmphibianTree from vertlife.org [Jetz and Pyron, 2018]. We report average *F* and

p values across all 1,000 correlations, and we depict trend lines based on the average slope and y-intercept across all correlations. To measure correlations among traits in the auditory system of species independent from body size, we calculated the residuals for each species in these PGLS models with body size (again, averaged over all 1,000 trees). Because these residuals from PGLS models already incorporate phylogenetic relationships, and because of small sample sizes [≤20; Mundry, 2014], we ran linear models for the analyses comparing sensitivity residuals and morphological residuals.

Results

Body Size and Auditory Measurements

We first confirmed that body size correlated with the mating call's dominant frequency and morphological measures of the auditory system across frogs. Indeed, we found significant correlations between our behavioral measure (dominant frequency (DF); Fig. 1c) and our three morphological measures (TA, APH and BPH; Fig. 1d, e). These correlations were significant when analyzed with phylogenetically controlled models (PGLS models: DF: $r^2 = 0.24$, $F_{1,64}$ =, 20.6, $p < 0.0001$; TA: $r^2 = 0.46$, $F_{1,56} = 48.3$, $p < 0.0001$; APH: $r^2 = 0.17$, $F_{1,46} = 9.4$, $p = 0.0041$; BPH: $r^2 = 0.18$, $F_{1,40} = 9.1$, $p = 0.0065$). We also observed a weakly significant relationship between the APH and BPH counts ($r^2 = 0.11$, $F_{1,40} = 4.5$, $p = 0.0483$).

Our analyses of hearing sensitivity are complicated by the fact that there are a variety of methods used to determine sensitivity thresholds. Behavioral responses to conspecific call playbacks may be best for identifying biologically meaningful differences in hearing sensitivity; however, only a handful of species have been studied in this manner (Fig. 2a). In contrast, many species have been studied using neural responses to sound playback, and these methods often reveal sensitivity peaks in both a low-frequency range (Fig. 2b) and a high-frequency range (Fig. 2c), corresponding to the AP and BP sensitivities, respectively. We confirmed previous observations that measures of auditory brain stem responses (ABRs) tended to have higher thresholds [Taylor et al., 2019]. Therefore, for our single sensitivity score per species, we averaged across studies of neural responses, excluding those using ABRs. However, we note that ABRs represent a less invasive method where ongoing expanded use may soon provide opportunities for cross-species comparisons [Goutte et al., 2017; Womack et al., 2018; Yang et al., 2019; Lauridsen et al., 2020; Sun et al., 2020]. Figure 2d, e depict this average value per species against SVL for low and high-frequency sensitivity, respectively. Both sensitivities were significantly negatively correlated with SVL (PGLS; low frequency: $r^2 = 0.26$, $F_{1,29} = 10.5$, $p = 0.0034$; high frequency: $r^2 = 0.36$, $F_{1,31} = 17.7$, $p = 0.0002$) indicating that larger frogs tend to be more sensitive to sound. We also observed that the low and high-frequency sensitivity in the same species were correlated, and that species tended to be more sensitive at their low-frequency peak than their high-frequency peak (Fig. 1f; PGLS: $r^2 = 0.45$, $F_{1,28} = 23.2$, $p < 0.0001$).

Overall, these analyses confirm that body size is correlated with measures of the acoustic communication system in frogs across behavioral, morphological, and neurological aspects of the system.

Sensitivity and Morphology

First, we asked whether morphological variation is related to variation in sensitivity across species. We found a significant correlation between TA and sensitivity for both sensitivity peaks (Fig. 2g, h; PGLS: $r^2 = 0.32$, $F_{1,18} = 8.0$, $p = 0.0090$). We found a nonsignificant negative trend for the relationship between AP hair cell counts and low frequency sensitivity (Fig. 2i; PGLS: $r^2 = 0.46$, $F_{1,5} = 4.4$, $p = 0.0934$), but no significant relationship between BP hair cell counts and high frequency sensitivity (Fig. 2i; PGLS: $r^2 = 0.18$, $F_{1,7} = 1.6$, $p = 0.2510$).

Next, we accounted for body size and asked whether changes to morphological characters, independent from changes to body size, could relate to the sensitivity of different species of frog. Figure 3a depicts the correlation between the body size residuals of the TA and the body size residuals of low-frequency sensitivity. For example, points in the upper left quadrant indicate species that have a smaller TA than expected based on their body size, and also are less sensitive (i.e., have a higher dB threshold sensitivity value) than expected based on their body size. This relationship was not statistically significant (linear model: $r^2 = 0.02$, $F_{1,15} = 0.2$, $p = 0.6355$), nor was the relationship with high frequency sensitivity (Fig. 3b; linear model: $r^2 = 0.01$, $F_{1,18} = 0.2$, $p = 0.6945$). We then compared hair cell counts to sensitivity. We observed a nonsignificant trend between the residuals for AP hair cell counts and sensitivity, but not for BP hair cell counts (Fig. 3c, d; APH: $r^2 = 0.50$, $F_{1,5} = 5.0$, $p = 0.0749$; BPH: $r^2 = 0.21$, $F_{1,7} = 1.8$, $p = 0.2209$). There was a nonsignificant negative relationship between the APH and BPH residuals ($r^2 = 0.1$, $F_{1,40} = 2.5$, $p = 0.1185$).

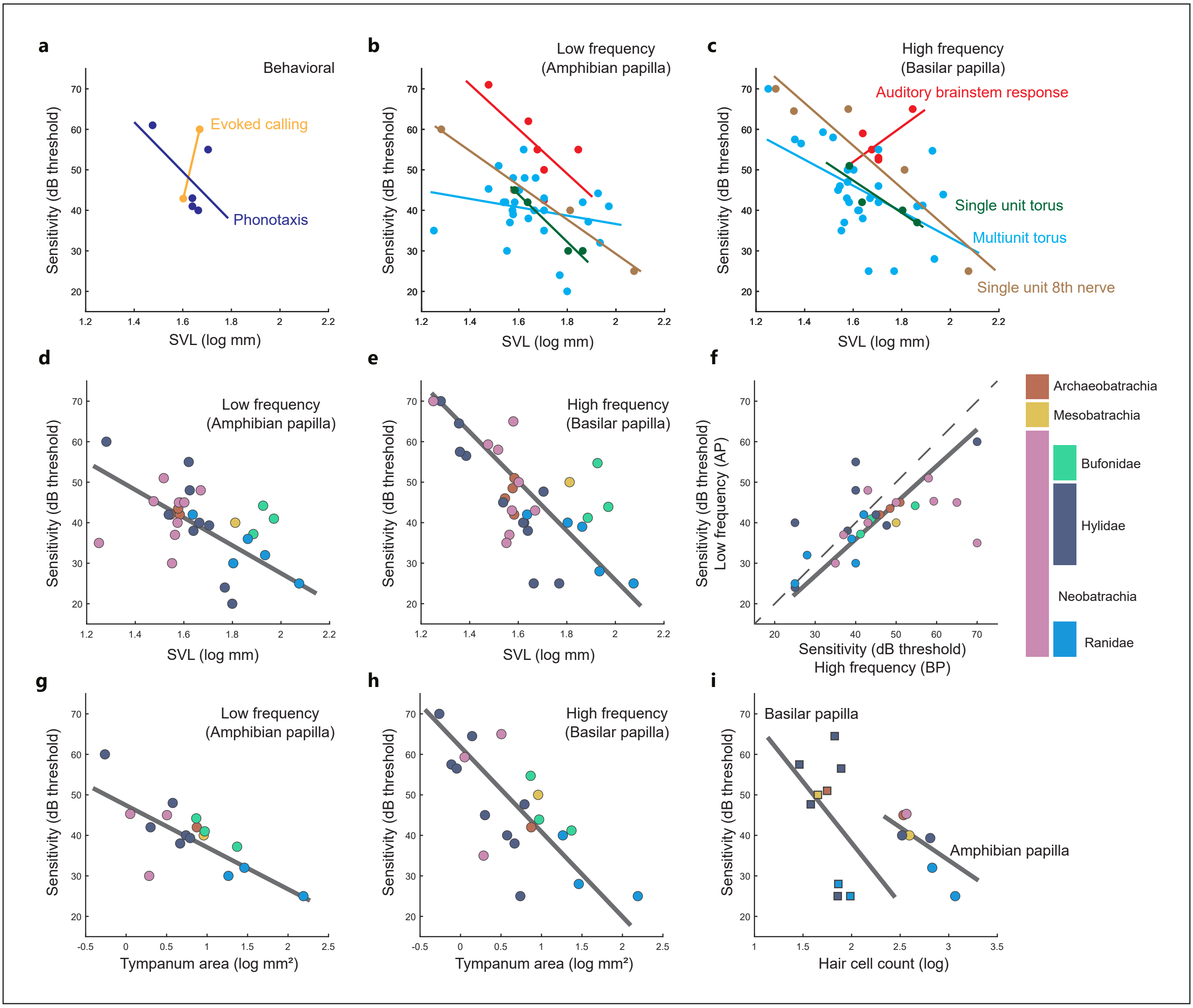

Fig. 2. Sensitivity varies by method and correlates with body size and morphology. **a** Scatterplots and simple linear correlations for two behavioral measures of auditory sensitivity. **b** Scatterplots and simple linear correlations for neural measures of low-frequency auditory sensitivity. **c** Scatterplots and simple linear correlations for neural measures of high-frequency auditory sensitivity. Colors differentiate each of four methods and are the same as those in **b**. **d**, **e** The significant negative relationship between SVL and low (**d**) or high (**e**) frequency sensitivity. **f** The significant relationship between low and high-frequency sensitivity. The fact that most points are below the line of unity (dashed line) illustrates the trend for better sensitivity at low frequencies. **g–i** Correlations between sensitivity and measures of morphology: tympanum area and low-frequency sensitivity (**g**), tympanum area and high-frequency sensitivity (**h**), amphibian papilla hair cell counts and low-frequency sensitivity (**i**; circles) and basilar papilla hair cell counts and high-frequency sensitivity (**i**; squares). For **d–g**, the trend line depicts the output of a phylogenetically controlled correlation, and the color of the points corresponds to the species diagram to the right and Figure 1b.

Calling Behavior and Morphology

Finally, we asked whether the same aspects of morphology (TA and hair cell counts) could relate to the calling behavior by examining the dominant frequency (DF) of the males' mating calls. In particular, the two frog papillae, AP and BP, tend to respond best to lower and higher frequencies, respectively [Wilczynski and Capranica, 1984]. Therefore, the hair cell counts in these papillae might relate to DF. Again, to account for body size, we used residuals for morphological measurements from the

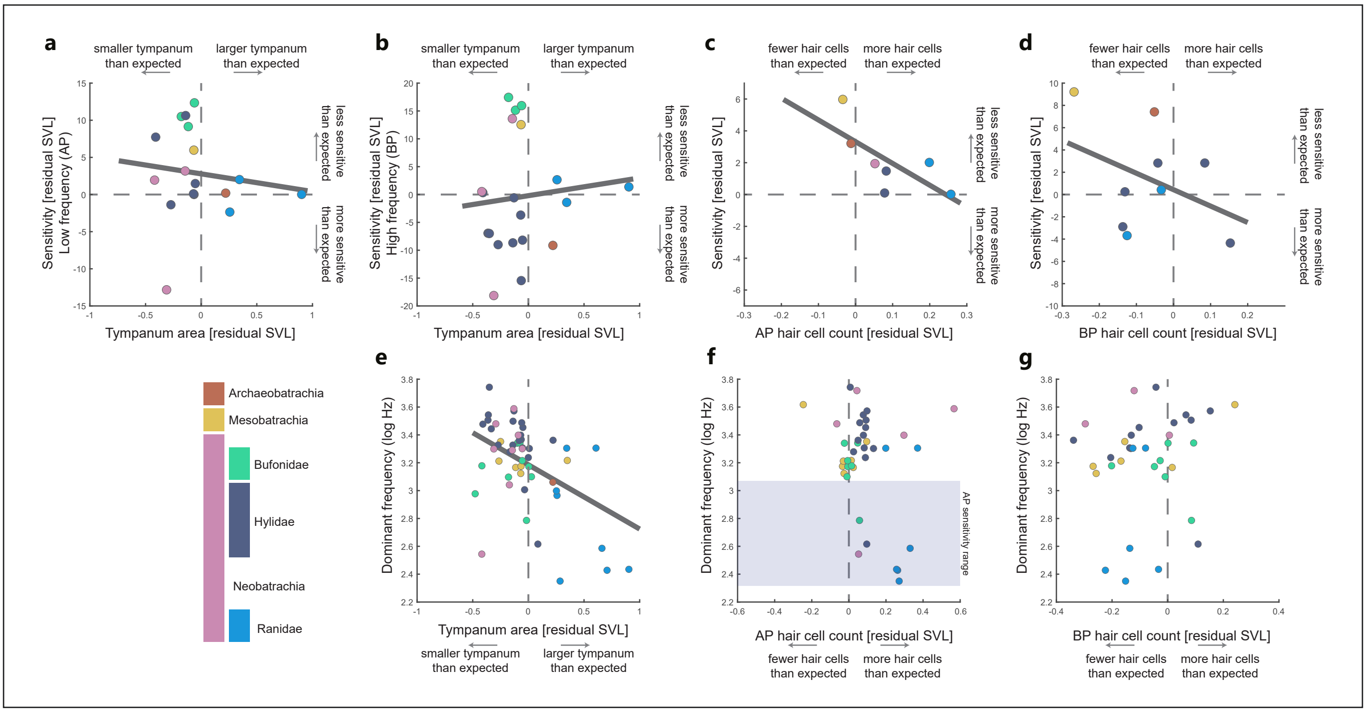

Fig. 3. Correlations between features of the acoustic communication system after accounting for body size. **a–d** Correlations between morphology and sensitivity, independent of body size. All measures plotted here are the residuals from the phylogenetically controlled (PGLS) correlations with snout-vent length (SVL). Depicted are the correlations predicting low-frequency auditory sensitivity by tympanum area (**a**), high-frequency auditory sensitivity by tympanum area (**b**), low-frequency auditory sensitivity by amphibian papilla (AP) hair cell count (**c**), and high-frequency auditory sensitivity by basilar papilla (BP) hair cell count (**d**). **e–g** Evidence that behavior (mating call dominant frequency) and morphology can correlate independent of SVL. Plotted are the correlations between dominant frequency and tympanum area (**e**), AP hair cell count (**f**), and BP hair cell count (**g**), with all morphological measures represented by the residuals from PGLS analysis with SVL. For **a–g**, points are color-coded based on the phylogeny diagram on the bottom left and in Figure 1b.

correlation with SVL. However, we used the actual dominant frequency value from each species.

Overall, we found a significant correlation between dominant frequency and TA, but not with either of the hair cell counts (Fig. 3d, f; PGLS models: TA: $r^2 = 0.11$, $F_{1,51} = 6.4$, $p = 0.0174$; APH: $r^2 = 0.01$, $F_{1,36} = 0.5$, $p = 0.5233$; BPH: $r^2 = 0.00$, $F_{1,32} = 0.0$, $p = 0.9096$). Specifically, we found that frogs with a larger tympanum than expected for their body size tended to have lower dominant frequencies, which appears to be driven largely by members of the Ranidae family. In addition, we found that the seven species with a dominant frequency within the sensitivity range of the AP (< 1,200 Hz) tended to have more AP hair cells than expected for their body size compared to species with dominant frequencies above the range of the AP. Indeed, using a linear model we found that frogs with low DF (< 1,200 Hz) had higher residual APH values ($r^2 = 0.11$, $F_{1,36} = 4.4$, $p = 0.0420$); however, this result on very unbalanced data was not borne out using a PGLS analysis ($r^2 = 0.0$, $F_{1,36} = 0.05$, $p = 0.8622$). There exists much more variation in the sensitivity range of the BP, with the peak sensitivity generally correlating with the mating call DF [Gerhardt and Schwartz, 2001; Richards, 2006]. However, we did not observe any clear relationship between DF and BPH.

Discussion

How functionally related traits scale with body size has been of interest to biologist dating back at least to Huxley [1932]. Acoustic communication, in particular, requires morphological and neurological coordination both within and between senders and receivers. Scaling relationships between phenotypic traits and body size (allometry) are mechanisms that could facilitate such functional correlations. However, it remains unclear the degree to which traits can exhibit correlated evolution beyond that expected by phylogenetic relationships and constraints from body size. There are numerous sources of selection acting on animals (e.g., sexual selection, predation, physiological constraints) meaning that a change in body size due to predation, for example, could lead to ripple effects that alter other aspects of vocal communication systems.

Here, we present novel evidence across anuran species for associations between morphology and auditory sensitivity, as well as morphology and calling behavior using analyses that account for body size and control for phylogenetic relatedness. First, we confirmed previous reports that body size (SVL) correlates with measures of auditory morphology, auditory sensitivity, and dominant frequency. Importantly, we are unaware of any previous studies in frogs demonstrating that body size predicts auditory morphology or auditory sensitivity with phylogenetically controlled (PGLS) analyses, correlations suggested by previous comparative work [Loftus-Hills, 1973; Wilczynski et al., 1984; Fox, 1995]. We indeed show that independent from shared phylogenetic history, body size strongly predicts auditory morphology, sensitivity, and dominant frequency. Furthermore, we explored how different methodologies compared in their estimates of auditory sensitivity. We found broad support for a negative correlation with body size, regardless of method, as well as a reasonable concordance in the slope of the correlation across many of the methods used.

Leveraging the residuals from these PGLS analyses with SVL, we next asked whether auditory morphology could predict auditory sensitivity after controlling for body size and phylogeny. While limited by small sample sizes, we did observe that frogs with relatively high numbers of AP hair cells for their body size demonstrated a trend toward improved auditory sensitivity for their body size. We continued to leverage residuals from the PGLS analyses with SVL to ask whether aspects of auditory morphology could predict the dominant frequency of mating calls. We found that frogs with relatively large tympana for their body size also tended to have lower dominant frequencies, and larger tympana are likely to be more effective at perceiving lower frequencies [Chung et al., 1981; Heyer and de Carvalho, 2000; Bradbury and Vehrencamp, 2011]. We did not find any broad trends for AP or BP hair cell counts and dominant frequency. However, we did find limited support for the idea that species with dominant frequencies in the AP's sensitivity range have more AP hair cells for their body size than species with dominant frequencies outside the AP's sensitivity range. We also note that, while dominant frequency is important in frog communication, it only captures one measure of the rich acoustic structure of a frog's call. Indeed, some species have multiple frequency peaks in their call, which can act to stimulate the AP and BP independently [Gerhardt, 1976, 1981; Ryan and Rand, 1990; Ryan et al., 1990].

This study focused on the primary sound pathway to the brain and on morphological characters that are relatively fixed within a species. However, recent work has shown other factors that could influence sound sensitivity in frog receivers. For instance, neurological processing and a separate lung-mediated sound pathway can enhance the salience of species-specific calls [Lee et al., 2017, 2021]. In addition, sound sensitivity can covary with fluctuating hormone levels [Miranda and Wilczynski, 2009a; Baugh et al., 2019; Gall et al., 2019]. Many of the studies measuring auditory sensitivity used animals currently engaging in breeding behaviors where sensitivity is typically high; however, variation in methodology could account for some unexplained variation in this study. Further research on how the rich spectral components of calls, including ultrasonic components, relates to hearing sensitivity will also be of great interest [Feng et al., 2006].

Our analyses are generally limited by small sample sizes, which may have reduced our power to detect relationships between auditory morphology and auditory sensitivity after accounting for body size. While we had some data for many species of frogs ($n = 81$), there was unfortunately small overlap for some measures across species (i.e., only 7 species with both a low-frequency auditory sensitivity and AP hair cell measure). We hope that additional data collection, particularly in species where we already have either morphology or sensitivity data, can help clarify such relationships.

In the meantime, these results provide preliminary evidence that aspects of auditory morphology can evolve independently from body size, and influence perception and behavior. While Tonini et al. [2020] found "allometric escape" a rare event in the context of anuran body size and dominant frequency, we find that it may be more common in the context of sound sensitivity and morphology. The strongest evidence comes from the AP, where we observed a significant relationship between auditory sensitivity and hair cell counts after accounting for both body size and phylogenetic relatedness. This may be strongest in species with lower-frequency mating calls, where we tended to observe higher AP hair cell counts relative to body size. On the other hand, there is no question that functional correlations between various characters of the communication system can be maintained through scaling of body size. The roles of the AP and BP in promoting speciation in frogs remains a matter of debate [Ryan, 1986; Richards, 2006]. The fact that we find the AP as the region with a closer relationship to auditory sensitivity is surprising given that most frogs produce calls with dominant frequencies in the sensitivity range for the BP, as seen here and in previous studies [Richards, 2006].

We also observed variation across some of the major groupings of anurans. Of particular interest is the contrast between the two families Ranidae and Bufonidae. These two families contained the largest frogs in the dataset but differed strikingly in their TAs and AP hair cell counts (Fig. 1b, c), with Ranidae tending to have larger tympana and higher counts. Furthermore, Bufonindae had some of the least sensitive species, while Ranidae tended to be quite sensitive (Fig. 2c). Figure 3a demonstrates this contrast between these families with Bufonidae having low TA and low sensitivity for their body size, and Ranidae having large TA and high sensitivity for their body size. Unfortunately, we do not have both sensitivity and AP hair cell counts for any members of Bufonidae, but the group data suggest that a similar pattern would be observed in Figure 3b.

Overall, these data provide foundational results for how auditory morphology, sensitivity, and dominant frequency relate to one another using phylogenetically controlled analyses and while accounting for body size. Furthermore, the strong correlations of these features with body size demonstrate the constraints that body size and allometric scaling can have on aspects of communication systems, which can facilitate phenotypic coordination both within and between senders and receivers. These results also highlight the need for additional data collection in order to understand the full morphological, neurological, and behavioral diversity of the thousands of vocalizing frog species. Given the increasingly rapid rates of extinctions among frogs and the importance of vocal communication for reproductive success, the collection of such data has never been more important [Gerhardt, 1994; Wake and Vredenburg, 2009].

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Statement of Ethics

An ethics statement was not required for this study type as this study did not produce any original data from animals.

Conflict of Interest Statement

The authors have no conflicts to disclose.

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Author Contributions

M.J.R and L.S.J. conceived the project. L.S.J. gathered data, ran analyses, and created figures. L.S.J., M.J.R., R.C.T., and K.L.H. wrote the manuscript. M.J.R., R.C.T., and K.L.H. acquired the funding. M.J.R. supervised the project.

Data Availability Statement

Data used for analysis are available as online supplementary material.

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