

# Call acoustics reflect body size across four clades of anurans

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bioacoustics; allometry; anurans.

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## Abstract

An inverse relationship between body size and advertisement call frequency has been found in several frog species. However, the generalizability of this relationship across different clades and across a large distribution of species remains underexplored. We investigated this relationship in a large sample of 136 species belonging to four clades of anurans (*Bufo*, Hylinae, *Leptodactylus* and *Rana*) using semi-automatic, high-throughput analysis software. We employed two measures of call frequency: fundamental frequency (F0) and dominant frequency (DF). The slope of the relationship between male snout-vent length (SVL) and frequency did not differ significantly among the four clades. However, *Rana* call at a significantly lower frequency relative to size than the other clades, and *Bufo* call at a significantly higher frequency relative to size than *Leptodactylus*. Because the relationship between F0 and body size may be more straightforwardly explained by biomechanical constraints, we confirmed that a similar inverse relationship was observed between F0 and SVL. Finally, spectral flatness, an indicator of the tonality of the vocalizations, was found to be inversely correlated with SVL, contradicting an oft-cited prediction that larger animals should have rougher voices. Our results confirm a tight and widespread link between body size and call frequency in anurans, and suggest that laryngeal allometry and vocal fold dimensions in particular are responsible.

## Introduction

Among vertebrates, the relationship between call parameters and body size – ‘acoustic allometry’ – has been of particular interest for two reasons. First, body size imposes severe constraints on vocal signals, and well-understood physical principles can lead to tight relationships between body size and vocal parameters (e.g. Davies & Halliday, 1978; Jones, 1999; Fitch, 2000). Second, body size often plays a crucial role in selection and thus can be of great importance in mate choice and territoriality (e.g. Davies & Halliday, 1978; Asquith & Altig, 1990; Wagner, 1992; Fitch & Hauser, 2002). Therefore, acoustic allometry provides an excellent arena in which to investigate interactions between selection and constraints in vocal evolution.

An inverse relationship between body size and call frequency has been documented in numerous species of anurans (reviewed in Duellman & Trueb, 1994; Fitch & Hauser, 2002; Gerhardt & Huber, 2002), in most cases presumably because of a direct correlation between body size and the dimensions of the vocal folds (Martin, 1972; Nevo & Schneider, 1976; Ryan, 1988). However, the issue of how these within-species correlations generalize across larger numbers of clades has rarely been explored. A few studies conducted large-scale interspecific comparisons but used a moderate number of

species (Menzies & Tyler, 1977; Duellman & Pyles, 1983; Zimmerman, 1983; Cocroft & Ryan, 1995) or were restricted to one faunal area (Hoskin, James & Grigg, 2009). One difficulty is that broader taxonomic analyses require large volumes of data to be processed. Traditional time-consuming methods of acquiring such data (e.g. ‘eyeballing’ spectrograms in published papers) face problems of comparability across taxa (Hauser, 1993; Jones, 1999). The development of high-throughput, semi-automated analysis tools over the last decade, mostly in the context of speech and music processing, offers an attractive alternative to this traditional approach. Here, as a first exploration of the potential of this alternative approach, we investigate the relationships between body size and call frequency in a high-quality collection of 136 frog species belonging to four genera (*Bufo*, *Hyla*, *Leptodactylus* and *Rana*), which collectively are distributed on five continents (South America, North America, Europe, Asia and Africa) and in a wide variety of habitats.

The species in our sample were identified as belonging to one of these four genera on the basis of their identification on CD recordings. Although some of the species assigned to *Hyla* on these recordings have since been reclassified to other genera, current taxonomies subsume these clades under the Hylinae subfamily, suggesting a close phylogenetic relationship. Here, we follow the nomenclature used by Pyron & Wiens

(2011), which is more conservative than that of Frost (2011), especially regarding *Bufo* and *Rana* that mostly retain the composition that they had prior to Frost *et al.* (2006). However, given that some of the proposed revisions to the nomenclature remain controversial (Pauly, Hillis & Canatella, 2009) and that the generic content of certain families is still in flux, we chose to avoid referring specifically to 'genera' or 'subfamilies' and use the more generic term 'clades' for the remainder of the paper.

In this paper, we address the following issues. First, to what extent do within-species relationships between size and frequency generalize across species? Second, is this relationship constant across clades (specifically, are the slopes and intercepts the same)? Two more specific hypotheses require further explanation. The former concerns two different measures of 'call frequency'. The first of these, fundamental frequency (F0), corresponds physically to the vibration rate of the vocal folds, and there is a clear theoretical prediction that the lowest F0 producible will be determined by the length and mass of the vocal folds. The second measure, termed 'dominant frequency' (DF), is the frequency in the call spectrum with the highest amplitude. DF is easy to measure and thus commonly reported in the anuran literature, but its physical meaning is ambiguous. While it often corresponds to the F0, it could also denote a higher harmonic, or in the case of noisy calls even a formant frequency (a vocal tract resonance). Consequently, we measured both F0 and DF in this study to enable a direct comparison.

The latter hypothesis, due to Morton (1977), describes a more subtle relationship between body size and call acoustics. Morton proposed that larger vibrating masses, having more degrees of freedom, would be more likely to produce noisy broadband output. Thus, larger animals would have rougher, less tonal calls, and this would account for the common association between roughness and aggression (Morton, 1977; Hauser, 1993). The physical intuitions behind this proposal remain somewhat dubious (cf. Fitch & Hauser, 2002), but we nonetheless sought evidence for Morton's proposal by examining the relationship between tonality and body size in our multispecies sample. To our knowledge, Morton's hypothesis has not been tested using anurans' vocalizations. Here, we used spectral flatness (SF) (also known as tonality coefficient or Wiener entropy), an acoustic feature commonly used to describe animal vocalizations (Tchernichovski *et al.*, 2000), as a proxy for tonality. SF corresponds to the geometric mean of the power spectrum divided by its arithmetic mean (Dubnov, 2004), with a lower value representing a more 'spiky' spectrum, indicating the presence of partials (but not necessarily periodicity).

## Materials and methods

### Recordings and acoustical analysis

Recordings were obtained from commercially available CDs (see Supporting Information Appendix S1) comprising anurans' vocalizations from North, South and Central America, Europe, South Africa, Japan and Korea. One file per

species was digitally transferred to WAV files at a sampling rate of 44.1 Hz for further processing.

For each species, the longest continuous sequence containing only advertisement calls of a lone male was extracted using the PRAAT software, version 5.1.44 (Boersma & Weenink, 2009). Recordings that did not contain at least one sequence clearly comprising vocalizations of a single male were not used. In total, 18 such recordings were discarded.

The acoustical analysis was conducted using the MIR Toolbox 1.3.2 in MATLAB (Lartillot, Toivainen & Eerola, 2008). Recordings were first converted from stereo to mono. Acoustical parameters were analyzed using a window of 40 ms with step-size of 20 ms and a 2048-point Fourier window size (fast Fourier transform window size).

Several recordings contain a significant proportion of quiet frames, during which the vocalization of interest is not emitted. Because these low-energy segments may affect the validity of the analysis (Seyerlehner *et al.*, 2009), they were excluded according to the following rule: the root average of the square of the time-varying amplitude of the acoustic signals, that is, the root-mean-square energy (RMS), was calculated for each 40-ms frame, and frames for which the RMS energy was less than 15% of the maximum energy of the analyzed sound sample were removed. The threshold value of 15% was chosen because higher values did not increase the accuracy of a classification algorithm developed to sort these vocalizations by clade, whereas lower values led to a decrease in classification accuracy (Gingras and Fitch, unpubl. data).

Recordings for which less than 50 frames (corresponding to 2 s of audio signal) remained after removing the quiet frames were excluded from further analysis. In total, 17 such recordings were excluded. Two additional recordings were also discarded because of an overall poor signal-to-noise ratio.

Recordings from 136 species were retained, including 38 calls from species belonging to the *Bufo* genus, 40 from *Hyla*, 25 from *Leptodactylus* and 33 from *Rana*, according to the nomenclature used in the CD notes. A total of 53 recordings were made in North and Central America, 52 in South America, 16 in East Asia, 9 in South Africa and 6 in Europe. A complete list of the 136 species, including their scientific names as listed on the CD recordings, as well as their revised scientific names according to the nomenclature proposed by Pyron & Wiens (2011) and that proposed by Frost (2011), is provided in Supporting Information Appendix S1.

DF, F0 and SF were computed for each 40-ms frame. F0 was computed using the autocorrelation algorithm proposed by Boersma (1993), as implemented in the MIR Toolbox. F0 values were retained for calls for which F0 could be estimated for at least 10 frames (125 out of 136 species).

Temperature data were available for some of the recordings and were provided in Supporting Information Appendix S1. These data were sparse and were not analyzed in the present study. In any case, temperature-induced effects on the acoustical properties of calls are presumably relatively small in comparison to taxonomical or ecological influences (Hoskin *et al.*, 2009). Moreover, DF has rarely been shown to be affected by temperature (Gerhardt & Huber, 2002).

**Table 1** Linear regression between base 10 logarithm of snout-vent length and base 10 logarithm of dominant frequency

Group	logDF				$R^2$	$F$	d.f.	$P$
	OLS		OLS-bisector					
	$\beta$	$\alpha$	$\beta$	$\alpha$				
All species	-1.072	4.997	-1.402	5.558	0.570	177.54	134	<0.001
<i>Bufo</i>	-0.889	4.788	-1.158	5.272	0.583	50.24	36	<0.001
Hyliinae	-1.032	4.969	-1.343	5.439	0.578	51.97	38	<0.001
<i>Leptodactylus</i>	-0.964	4.789	-1.337	5.444	0.502	23.17	23	<0.001
<i>Rana</i>	-1.098	4.876	-1.676	5.914	0.384	19.36	31	<0.001

Slopes and intercepts are provided for both OLS and OLS-bisector regression models (see the Methods section).

$\alpha$ , y-intercept;  $\beta$ , slope; d.f., degrees of freedom;  $F$ ,  $F$ -ratio; logDF, log dominant frequency; OLS, ordinary least squares;  $P$ ,  $P$ -value;  $R^2$ , proportion of variance explained.

Snout-vent length (SVL) was used as a proxy for male body size. Supporting Information Appendix S1 provides the median male SVL for all 136 species comprising the data analyzed here, as well as the sources used to obtain those data.

### Statistical analyses

Statistical analyses were performed in MATLAB R2010b and in SPSS version 19 (SPSS Inc., Chicago, IL, USA). Each variable (SVL, DF, F0 and SF) was tested for normality and homoscedasticity among all four clades. A log-transformation was necessary to achieve normality for SVL, DF and SF, resulting in the derivative variables logSVL, logDF and logSF. Base 10 logarithmic transformations were used to facilitate the interpretation of the transformed variables. For F0, a square root transformation was necessary to achieve normality, yielding sqrtF0 (the log-transformation did not achieve normality in this case).

For each of these acoustic parameters, linear regressions were conducted to assess the relationship between body size and the acoustic features. Because there is uncertainty in both the predictor and the response variables, a model II linear regression analysis is appropriate here. Ordinary least squares (OLS) bisector regression, which has been shown to be the least biased type of model II regression (Isobe *et al.*, 1990) and has been increasingly used in allometric studies (Schmid, Tokeshi & Schmid-Araya, 2000), was used. However, because the statistical tests ( $F$ -ratio) are based on a model I (OLS) regression, both OLS and OLS-bisector regression models are presented.

Additionally, analyses of covariance (ANCOVAs) were performed to test for differences in the relationship between body size and the dependent variable (acoustic feature) among all four clades. Outliers were defined as species whose residuals were more than 3 SD away from the regression line.

## Results

### Relationship between DF and body size

A linear regression model showed a significant inverse relationship between logDF and logSVL across all 136 species and within each of the four clades (Table 1; Fig. 1). No outliers were observed.

We also sought to assess whether the relationship between DF and SVL differ among the four clades. In order to test whether the slopes were homogeneous among all clades, we included the interaction term in the ANCOVA; the interaction was not significant (ANCOVA common slopes model: logSVL  $\times$  clade  $F_{3,128} = 0.252$ ,  $P = 0.860$ ), indicating that the slopes can be treated as homogeneous. The model was run again without the interaction term, showing a significant relationship between logSVL and logDF ( $\beta = -0.986$ ,  $F_{1,131} = 136.07$ ,  $P < 0.001$ ), but different y-intercepts among the clades ( $F_{3,131} = 17.67$ ,  $P < 0.001$ ). Univariate contrasts confirmed that *Rana* have a lower logDF with respect to logSVL than *Bufo* ( $F_{1,131} = 49.75$ ,  $P < 0.001$ ), Hyliinae ( $F_{1,131} = 23.02$ ,  $P < 0.001$ ) and *Leptodactylus* ( $F_{1,131} = 11.24$ ,  $P = 0.001$ ), and that *Bufo* have a higher logDF with respect to logSVL than *Leptodactylus* ( $F_{1,131} = 9.26$ ,  $P = 0.003$ ). Adjusted means did not differ significantly among other clades.

### Relationship between DF and F0

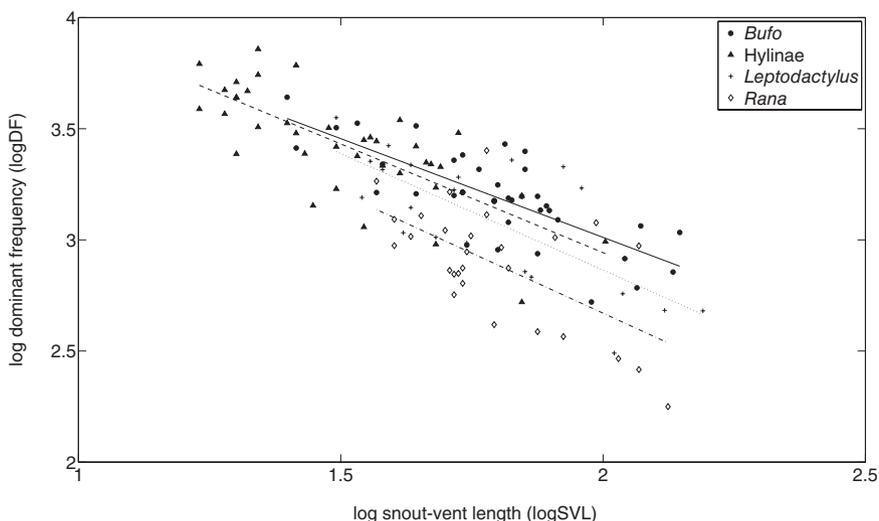
A linear regression model indicated a significant relationship between sqrtF0 and logDF across all 125 species for which F0 could be accurately determined,  $R^2 = 0.689$ ,  $F_{1,123} = 272.63$ ,  $P < 0.001$ . One outlier was found, *Hyla wrightorum*, whose call displays a much lower F0 than would be expected given its DF (see Fig. 2). In this case, the measured F0 likely corresponds to the pulse rate, which has been shown to be uncorrelated with SVL for this species (Sullivan, 1986).

As shown in Fig. 2, F0 is nearly identical to DF for the great majority of the species in our sample, with a minority (mostly Hyliinae) for which DF corresponds to the second harmonic (Cocroft & Ryan, 1995). It should be noted that DF was directly computed from the power spectrum, whereas F0 was obtained using the autocorrelation method.

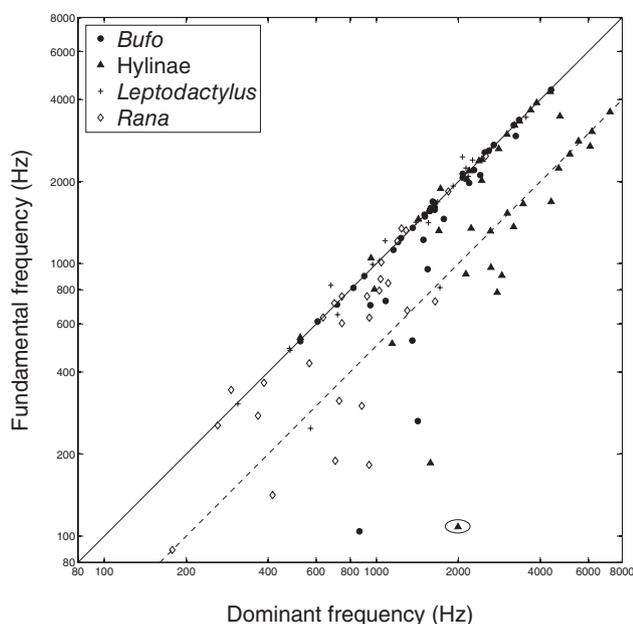
### Relationship between F0 and body size

A linear regression model showed a significant inverse relationship between sqrtF0 and logSVL across the 125 species for which F0 could be estimated and within each of the four clades (Table 2). No outliers were found.

The slope of the relationship between logSVL and sqrtF0 did not differ among the four clades (ANCOVA common



**Figure 1** Relationship between dominant frequency and body size across 136 frog species. The lines represent the mean ordinary least squares regression line for each clade: *Bufo* – solid line, Hylinae – dashed line, *Leptodactylus* – dotted line, *Rana* – dashed-dotted line. DF, dominant frequency; SVL, snout-vent length.



**Figure 2** Relationship between dominant frequency and fundamental frequency (a log-log scale was used for visual clarity). The solid line corresponds to a 1:1 ratio between dominant frequency (DF) and fundamental frequency (F0); the dashed line corresponds to a 2:1 ratio (meaning that the DF corresponds to the second harmonic in that case). The point corresponding to *Hyla wrightorum*, an outlier in the linear regression model predicting sqrtF0 from logDF, is circled.

slopes model:  $\log\text{SVL} \times \text{clade } F_{3,117} = 0.88, P = 0.451$ ). Removing the interaction revealed a significant relationship between  $\log\text{SVL}$  and  $\text{sqrtF0}$  ( $\beta = -46.43, F_{1,120} = 114.38, P < 0.001$ ), but different  $\gamma$ -intercepts among the clades ( $F_{3,120} = 14.72, P < 0.001$ ). Univariate contrasts revealed that *Bufo* have a higher  $\text{sqrtF0}$  with respect to  $\log\text{SVL}$  than Hylinae ( $F_{1,120} = 15.44, P < 0.001$ ) and *Rana* ( $F_{1,120} = 37.77, P < 0.001$ ), and also that

*Leptodactylus* have a higher  $\text{sqrtF0}$  with respect to  $\log\text{SVL}$  than Hylinae ( $F_{1,120} = 6.36, P = 0.013$ ) and *Rana* ( $F_{1,120} = 18.11, P < 0.001$ ). Adjusted means did not differ among other clades.

To assess whether  $\log\text{SVL}$  was more strongly correlated to  $\log\text{DF}$  or to  $\text{sqrtF0}$  for the 125 species for which F0 could be determined, we conducted a comparison of the Pearson correlation coefficients between  $\log\text{SVL}$  and  $\log\text{DF}$ , on one hand ( $r_{\text{DF}} = -0.760$ ), and between  $\log\text{SVL}$  and  $\text{sqrtF0}$ , on the other hand ( $r_{\text{F0}} = -0.678$ ), using the procedure described by Steiger (1980). The comparison yielded a  $Z$ -value of 1.90, which did not reach significance ( $P = 0.057$ , two-tailed), although it suggested a marginal tendency for  $r_{\text{DF}}$  to be larger (in absolute terms) than  $r_{\text{F0}}$ .

### Relationship between SF and body size

A linear regression model revealed a significant inverse relationship between  $\log\text{SF}$  and  $\log\text{SVL}$  across all 136 species and for two out of four clades (Hylinae and *Leptodactylus*), with a marginal tendency in the same direction found for *Rana*. *Bufo* was the only clade for which this relationship was not observed (Table 3; Fig. 3). No outliers were found.

The slope of the relationship between  $\log\text{SVL}$  and  $\log\text{SF}$  differed significantly among the four clades (ANCOVA common slopes model:  $\log\text{SVL} \times \text{clade } F_{3,128} = 2.86, P = 0.040$ ). Moreover, the clade term was also significant ( $F_{3,128} = 3.73, P = 0.013$ ), indicating significantly different  $\gamma$ -intercepts among the clades.

To assess the relationship between  $\log\text{SF}$  and  $\log\text{DF}$  while controlling for the effect of body size (expressed as  $\log\text{SVL}$ ) on the response variables, a partial correlation (Pearson) was conducted between  $\log\text{SF}$  and  $\log\text{DF}$ , with  $\log\text{SVL}$  as control variable, yielding a nonsignificant correlation of  $-0.021$  (d.f. = 133,  $P = 0.808$ ). A similar result was obtained when testing for the correlation between  $\log\text{SF}$  and  $\text{sqrtF0}$  (for the 125 samples for which F0 could be computed) with  $\log\text{SVL}$  as control variable ( $r = -0.048$ , d.f. = 122,  $P = 0.593$ ).

**Table 2** Linear regression between base 10 logarithm of snout-vent length and square root of fundamental frequency

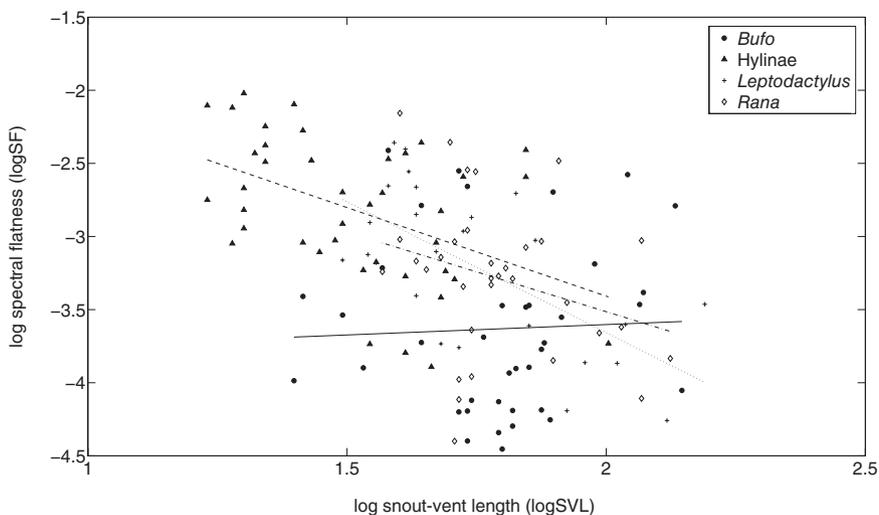
Group	sqrtF0		OLS-bisector		$R^2$	$F$	d.f.	$P$
	OLS		$\beta$	$\alpha$				
	$\beta$	$\alpha$						
All species	-41.20	106.51	-56.44	132.47	0.460	104.73	123	<0.001
<i>Bufo</i>	-47.13	122.67	-62.54	150.52	0.507	37.04	36	<0.001
Hylinae	-53.27	122.68	-68.96	146.28	0.545	43.12	36	<0.001
<i>Leptodactylus</i>	-45.67	117.67	-57.57	138.8	0.587	26.97	19	<0.001
<i>Rana</i>	-31.60	81.82	-50.54	115.80	0.251	8.70	26	0.007

Slopes and intercepts are provided for both OLS and OLS-bisector regression models (see the Methods section).  $\alpha$ , y-intercept;  $\beta$ , slope; d.f., degrees of freedom;  $F$ ,  $F$ -ratio; sqrtF0, square root of fundamental frequency; OLS, ordinary least squares;  $P$ ,  $P$ -value;  $R^2$ , proportion of variance explained.

**Table 3** Linear regression between base 10 logarithm of snout-vent length and base 10 logarithm of spectral flatness

Group	logSF		OLS-bisector		$R^2$	$F$	d.f.	$P$
	OLS		$\beta$	$\alpha$				
	$\beta$	$\alpha$						
All species	-1.374	-0.890	-2.384	0.828	0.230	39.94	134	<0.001
<i>Bufo</i>	0.143	-3.887	1.137	-5.672	0.002	0.07	36	0.795
Hylinae	-1.211	-0.985	-2.177	0.474	0.210	10.07	38	0.003
<i>Leptodactylus</i>	-1.788	-0.082	-2.578	1.301	0.419	16.57	23	<0.001
<i>Rana</i>	-1.089	-1.336	-2.297	0.830	0.086	2.90	31	0.098

Slopes and intercepts are provided for both OLS and OLS-bisector regression models (see the Methods section).  $\alpha$ , y-intercept;  $\beta$ , slope; d.f., degrees of freedom;  $F$ ,  $F$ -ratio; logSF, log spectral flatness; OLS, ordinary least squares;  $P$ ,  $P$ -value;  $R^2$ , proportion of variance explained.



**Figure 3** Relationship between spectral flatness and body size across 136 frog species. The lines represent the mean ordinary least squares regression line for each clade: *Bufo* – solid line, Hylinae – dashed line, *Leptodactylus* – dotted line, *Rana* – dashed-dotted line. SF, spectral flatness; SVL, snout-vent length.

## Discussion

The analyses presented here confirm and extend earlier results showing an inverse relationship between the DF of the advertisement calls of frogs and male body size. Notably, we demonstrate that this relationship extends to four anuran clades exhibiting a global distribution, on a large sample of 136

species. These results are in line with other studies reporting body size constraints on vocalization frequencies across species in insects (Bennet-Clark, 1998), birds (Ryan & Brenowitz, 1985), primates (Hauser, 1993) and bats (Jones, 1999), and suggest the existence of a broad, cross-taxon relationship between source dimensions (e.g. vocal fold or other source vibrator mass, e.g. syringeal membrane, size), body size and

call frequency (Fitch & Hauser, 2002). Furthermore, our results indicate that DF is a close approximation of F0 for most species and that the relationship between logSVL and sqrtF0 also holds for all four clades.

Although the strength of the relationship between logDF and logSVL was similar among the four clades, our analysis showed that *Rana* call at a significantly lower frequency relative to size than the other clades, and *Bufo* call at a significantly higher frequency relative to size than *Leptodactylus*. Other clades have been shown to differ in their relationship between body size and call frequency (Hoskin *et al.*, 2009). The mechanism of sound production in anurans involves the trunk muscles, the laryngeal anatomy and composition, and the oral cavity and vocal sacs. These components may function differently among various taxa, even though the mechanism is basically similar. Thus, most species of ranids and leptodactylids have median throat vocal sacs, whereas hylids have paired lateral vocal sacs and bufonids generally show reduced or, in some cases, non-functional vocal sacs (Wells, 2007). These differences may plausibly be related to differences in the strength of the relationship between body size and call acoustics.

SF, a quantitative measure of tonality, was also found to be inversely related to body size, with larger frog species generally displaying more tonal vocalizations (and thus lower SF values) than smaller species. The relationship holds globally for the sample of 136 species considered here, as well as for two of the four clades examined (Hylinae and *Leptodactylus*), with a marginal tendency in the same direction observed for *Rana*. To our knowledge, this is the first published study showing any relationship between SF and SVL in anurans. This finding stands in direct contradiction to the prediction of Morton (1977), which states that 'roughness' should increase with body size, given that larger frog species generally have lower frequency calls (see also Hauser, 1993). As with calling frequencies, differences in vocal sac type might have an influence on SF. For instance, the often-reduced vocal sac observed in bufonids (Wells, 2007) may play a role in the acoustic features of their calls, and may possibly be related to the absence of a relationship between logSF and logSVL noted in *Bufo*.

The findings of the current study raise interesting questions for our understanding of the role of vocal production mechanisms and the evolution of vocalization in vertebrates. First, the robust and consistent correlations we found between body size and vocalization frequencies stand in contrast to *intraspecific* acoustic allometry, where a relationship between size and call frequency has been found in some species (e.g. Davies & Halliday, 1978) but not in others (Sullivan, 1984; Asquith & Altig, 1990). This suggests that the development and anatomy of the larynx is consistent and constrained, enough to reveal strong physically based correlations across a large range of body sizes (in this study, from 1 g for *Hyla allenorum* to 300 g for *Bufo marinus*). Within a species, in contrast, body size range is smaller and anatomical modifications of the larynx, such as increase in relative larynx size in some individuals, or the addition of additional masses to the vocal folds (Gridi-Papp, Rand & Ryan, 2006) may play a larger role. Therefore,

cross-specific comparisons with large numbers of species provide a clear look at the fundamental physical constraints that can lead to acoustic cues to be informative about body size.

Second, we found evidence for more subtle shifts of the allometric relationship between clades (with *Bufo* and *Leptodactylus* calling at higher frequencies than Hylinae and *Rana*, when controlling for overall body size). These suggest that, within the broad constraints of physics, there is still room for adjustment of the relevant anatomical or physiological factors during evolution. The most obvious candidate factor is that, under selection for low frequencies, vocal fold length (and presumably laryngeal dimensions) could be increased, although only up to some limit. This prediction could be easily tested, given access to a large anatomical collection of frog larynges. The second possibility is behavioral, that by modifying vocal fold geometry or tension, individuals can quickly lower (or raise) their pitch without any concomitant changes in body size (Wagner, 1992; Bee, Perrill & Owen, 2000).

In contrast, the link we found between SVL and SF suggests that anatomical constraints may be more complex than previously thought, in that they influence not only acoustical features that may be directly derivable from the size of the vocal folds, such as DF and F0, but also features for which there is no obvious anatomical underpinning, such as SF. Further research will be necessary to identify potential mechanisms associated with this relationship and to verify whether it can be generalized to other species and potentially other taxa.

Another noteworthy finding in this study is that DF and F0, while closely related, are not identical. In most species, DF and F0 were the same, but in a significant number of cases, DF was associated with the second harmonic rather than the fundamental. In a handful of cases, there was no obvious relationship between the two. While the use of DF is commendable for its simplicity, and thus has a long history in studies of anuran vocalizations, these findings reveal a crucial limitation of the DF measure for large multispecies comparisons. From an acoustic viewpoint, it is invalid to equate the fundamental with the second harmonic, which is what scientists relying on DF might (unwittingly) be doing. Scientists seeking to understand the physical basis of acoustic allometry in anurans would thus be better off using F0, which corresponds directly to the vibration rate of the source, unlike the less reliable DF.

A possible limitation of the approach followed in the present study is that, whereas the advertisement calls were sampled from CD recordings, with only one male per species, the SVL measures obtained from the literature were averaged over many individuals and may not have been collected in the same region in which the recordings were made. Although this may have introduced some noise in the analysis, it is unlikely to significantly impact the general conclusions of our study, especially given that it is based on a large database and that it focuses on comparisons across clades. Nevertheless, follow-up studies may address this issue by sampling several recordings per species.

Finally, and most generally, our results illustrate the potential value of high-throughput, semi-automatic analysis

techniques for addressing broad taxonomic questions about constraints and adaptation in the evolution of vocal communication. To our knowledge, the number of species analyzed in the current study is unprecedented, and while the analysis proved time-consuming, it would have been far more protracted without the aid of modern high-throughput techniques.

## Acknowledgments

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## Supporting information

Additional Supporting Information may be found in the online version of this paper:

**Appendix S1.** List of recordings for the 136 species analyzed, with additional information about temperature and mean male snout-vent length.