



Sexual signals of the Amazonian frog *Allobates paleovarzensis*: geographic variation and stereotypy of acoustic traits

Igor Luis Kaefer* and Albertina Pimentel Lima

Coordenação de Pesquisas em Ecologia, Instituto Nacional de Pesquisas da Amazônia,
CP 478, 69011-970 Manaus, Amazonas, Brazil

*Corresponding author; e-mail: igorkaefer@hotmail.com

Accepted 3 January 2012

Abstract

Because of its close relationship with the process of evolutionary differentiation, it is expected that geographic variability in acoustic sexual traits should be greater among than within populations. This is particularly expected in organisms with typically high population genetic structure and low dispersal abilities, such as anuran amphibians. We studied the acoustic traits of the advertisement call in the small-sized dendrobatoid frog *Allobates paleovarzensis* through its range in Central Amazonia. We accessed the variability of call traits from the within-male to the among-population levels, and evaluated the degree of stereotypy of the call characteristics. Call variability had comparable magnitudes within and among populations, and was independent of the degree of stereotypy of call measurements. Therefore, none of the call traits stood out as a potential cue for discrimination between populations. Spectral call measurements were static and strongly related with body size, which explained between 30 and 35% of the variation of these acoustic traits. Temporal characters of the notes were dynamic and influenced by environmental temperature (e.g., 27% of note rate variation), whilst temporal measurements of the entire calls were not related to the co-factors analysed. Both spectral and temporal call traits varied among populations and between sides of the Amazon River. Our results also indicate that body size and sampling site jointly affected the variability of the call traits. However, geographic distances among populations and the river barrier had no significant effect on the overall acoustic variation, indicating that local stabilising selective forces may be important in the process of call differentiation.

Keywords

acoustic communication, advertisement call, Amazon River, biogeography, Dendrobatoidea, intraspecific variation, signal stereotypy.

1. Introduction

Variability in secondary sexual characters is a pivotal component that underlies the evolutionary framework in which selective forces take place, and changes in mate recognition systems may even precede the evolution of other indicators of specific status (Ryan et al., 1996; Panhuis et al., 2001). Therefore, analyses of geographic variation in intraspecific communication systems have the potential to detect the development of differentiation among populations, allowing researchers to derive testable predictions about the evolutionary significance of such divergences (Gerhardt & Huber, 2002).

An acoustic signal conveys information about its sender, such as species identity, body condition, and mate quality, and these characteristics have been decoded from the signals in a variety of animal groups, such as monkeys (Fischer et al., 2004), bats (Behr et al., 2007), crickets (Verburgt et al., 2011) and frogs (Chakraborty et al., 2010). Given that these signals are often related to sexual recognition and other aspects of pre-mating reproductive isolation (Gerhardt & Huber, 2002), their decomposition into their spectral and temporal properties should provide clues regarding their potential in sexual discrimination among populations (e.g., Pröhl et al., 2007; Gomez et al., 2011) and even individuals (e.g., Gasser et al., 2009; Bee et al., 2010; Melen-dez & Feng, 2010). Bioacoustic traits of individuals can be classified as static or dynamic according to the continuum of coefficients of variation observed (Gerhardt, 1991). Previous results suggested that female frogs impose more stabilising selection on stereotyped (static) properties, while directional selection should be imposed on more dynamic call traits, which show higher coefficients of variation (Gerhardt, 1991). In addition, the degree of stereotypy has been proposed to be related to the spatial pattern of variation of the different properties of the call (Castellano et al., 2000).

Spatial separation is a potential predictor of both gene flow and degree of environmental similarity between populations (Lougheed et al., 2006). Geographic signal differentiation is frequently observed in frogs, on both small (e.g., Boul et al., 2007; Rodríguez et al., 2010) and large scales (e.g., Heyer & Reid, 2003; Amézquita et al., 2009). Empirical patterns of spatial distribution of acoustic intraspecific variability have been attributed to different evolutionary forces which could act synergistically: correlations between acoustic and geographic distances are associated with neutral evolutionary processes such as genetic drift (Pröhl et al., 2007; Amézquita et al., 2009), while vicariant effects are invoked when differentiation in calls is congruent with the

crossing of geographic barriers (Simões et al., 2008; Rodríguez et al., 2010). Intraspecific signal divergence may reflect adaptation to structural features of the environment that differ between habitats (Ryan et al., 1990; Ryan & Wilczynski, 1991), or may be associated with female preferences; the latter may be interpreted as evidence of speciation caused by sexual selection (Boul et al., 2007; Guerra & Ron, 2008).

As a highly energy-demanding activity, the emission of advertisement calls has morphological and environmental constraints acting on ecological time scales, which are regarded as co-factors and should be controlled in comparative bioacoustic studies (Prestwich, 1994). They constitute an additional source of variation, of which the extent of interference varies between species and among the different acoustic traits considered (Gerhardt, 1991; Gerhardt & Huber, 2002). Studies observed a correspondence between the degree of call trait variability and the co-factors responsible for their variation (e.g., Castellano & Giacoma, 1998; Castellano et al., 2000), and our knowledge on the evolution of call trait stereotypy should be benefited by a better comprehension of this association.

Because of its close relationship — either as cause or outcome — with the process of evolutionary differentiation, it is expected that geographic variability in acoustic sexual traits should be greater among than within populations (e.g., Castellano et al., 2002; Smith & Hunter, 2005). This is particularly expected in organisms with typically high population genetic structure and low dispersal abilities, such as many anuran amphibians (Lougheed et al., 2006; Vences & Wake, 2007). The potential for a given call trait to act as a cue for discrimination (through either social recognition or sexual selection) can be evaluated by comparing the ratios between among- and within population coefficients of variation (Pröhl et al., 2007). Therefore, we analysed the variability in advertisement call traits throughout the distribution of the Amazonian lowland frog *Allobates paleovarzensis* (Dendrobatoidea: Aromobatidae) in order to access the variability of sexual signal in different levels. These are small-sized, diurnal, leaf-litter frogs that inhabit seasonally flooded forests of Central Amazonia (Lima et al., 2010). Dendrobatoid frogs are frequently used as models in both exploratory and experimental studies of acoustic communication, although no such studies have been conducted with any member of the dull-coloured *A. trilineatus* species group (sensu Morales, 2002). Related with comparing the within- and among-population variability in acoustic sexual traits, we aimed to evaluate the degree of stereotypy of

each call trait by assessing coefficients of variation for individual frogs, test the association between environmental temperature and male body size with acoustic characters, and test for the effect of a river barrier leading to call differentiation in the study system.

2. Material and methods

2.1. Data acquisition

We recorded advertisement calls of *Allobates paleovarzensis* in ten localities (Figure 1), which will be regarded as individual populations in this study. Sampling sites were located in the state of Amazonas, Brazil, on both sides of the Amazon River, and were at least 28 km distant from each other. This study extends the known distribution of this species, for which the confirmed occurrence was formerly restricted to its type locality in the municipality of

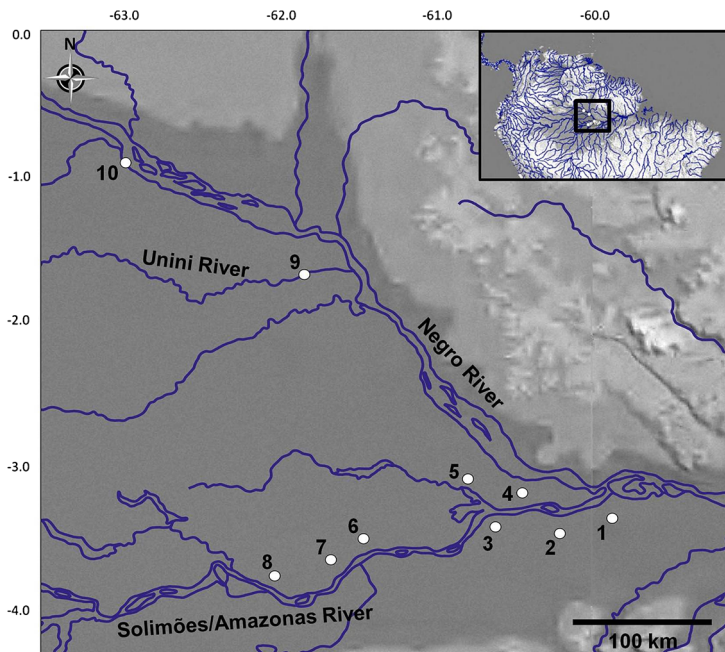


Figure 1. Geographic distribution of sampling locations in Central Amazonia. 1, Careiro; 2, Janauacá; 3, Manauquiri; 4, Hiléia; 5, Novo Airão; 6, Anamá; 7, Anori; 8, Codajás; 9, Unini; 10, Barcelos. This figure is published in colour in the online edition of this journal, which can be accessed via <http://www.brill.nl/beh>

Careiro da Várzea (Lima et al., 2010). Therefore, this study comprises the entire known range of *A. paleovarzensis*. Fieldwork was conducted between January 2009 and June 2010. In total, we recorded advertisement calls of 123 males, with a mean of 12 males per population ($SD = 3.8$, range = 2–15; 12–15 when excluding Unini). Each male had its advertisement call recorded for about three minutes with a Marantz PMD660 digital recorder (44.1 kHz sampling rate; 16-bit resolution) and a Sennheiser K6/ME66 directional microphone positioned approx. 1 m from the calling site. After each recording, we measured environmental (air) temperature with a digital thermometer, and captured the frog, to measure body size (snout–vent length, SVL, in mm) with a digital calliper. Voucher specimens were deposited in the Herpetological Collection of the Instituto Nacional de Pesquisas da Amazônia, in Manaus, Brazil.

Call measurements were obtained from recordings in Raven Pro 1.3 software (Charif et al., 2008). The advertisement call of *Allobates paleovarzensis* consists of groups of single notes separated by silent intervals (Lima et al., 2010; see Figure 2). For each recording, we chose 10 calls with less background noise for detailed analyses. Note measurements, including the spectral traits, were taken from the middle or next note of each call. Spectral measurements were taken after a Fast Fourier Transform with frequency resolution of 82 Hz and 2048 points. We considered ten call traits in our analyses: Note rate (NR, in notes/s); Note duration (ND, in s); Internote interval, as the silent interval between two consecutive notes of a call (InI, in s); Call rate (CR, in calls/s); Call duration (CD, in s); Intercall interval, as the silent interval between two consecutive calls (IcI, in s); Maximum (peak) frequency, as the frequency of higher intensity calculated for the entire call by a power spectrum function of Raven Pro 1.3 (MF, in Hz); Lowest frequency (LF, in Hz); Highest frequency (HF, in Hz); Note modulation, as the difference between the highest and the lowest frequencies of the call (NM, in Hz). LF and HF were measured at 20 dB below the peak intensity, the value at which the signal energy could still be clearly distinguished from background noise (Lima et al., 2010).

2.2. Data analysis

The data set was tested for normality. When appropriate, we log-transformed data prior to carrying out the parametric statistical analyses. Given that certain call traits are affected by environmental temperature and male body size

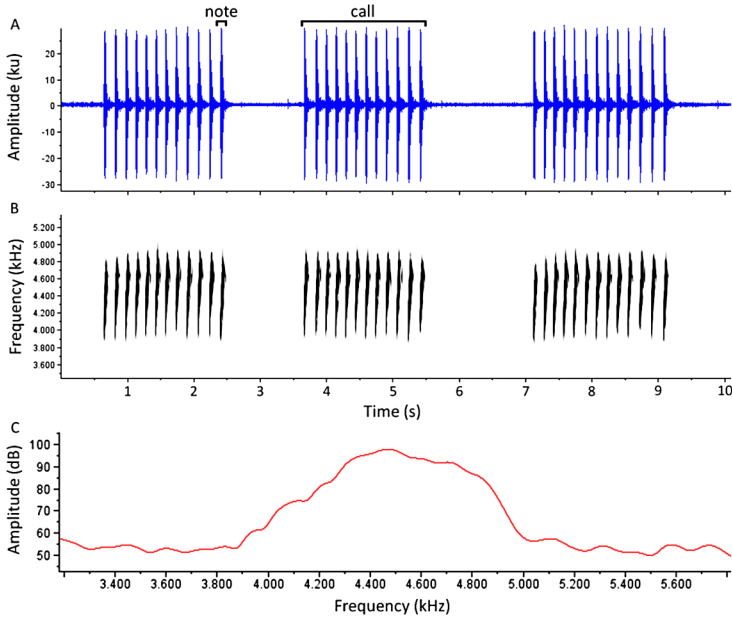


Figure 2. (A) Oscillogram and (B) spectrogram of three multinote advertisement calls, and (C) power spectrum of a single note of a male *Allobates paleovarzensis* at its type locality in Careiro da Várzea, Brazil. This figure is published in colour in the online edition of this journal, which can be accessed via <http://www.brill.nl/beh>

(Gerhardt & Huber, 2002), we tested to what extent these two co-variables affect each of the call parameters in the species studied. For temperature, linear regression analyses were conducted. For all within- and among-population analyses we used temperature-adjusted acoustic data, by calculating overall regression coefficients (b) for all call parameters at the mean temperature of 25.4°C (SD = 1.08, range = 23.1–28.0). We applied the equation $Y_{\text{adj}} = y - (b \times T_{\text{calling site}}) + (b \times T_{\text{mean}})$ where Y_{adj} is the adjusted value of call measurement, b the regression coefficient and T the temperature. To test for morphological correlates of call traits, we performed linear regression analyses between body size and temperature-adjusted call measurements. The adjusted r^2 values obtained were used to estimate the percentage of variation explained by the independent variable.

We calculated coefficients of variation ($CV = 100\% \times (SD/\bar{x})$) for each call trait within individuals, within populations, and over all individuals combined. For each focal male, we determined the individual mean (\bar{x}_{wi}) and standard deviation (SD_{wi}) of each call property ($n = 10$ calls/male), and we

used these values to calculate a within-individual coefficient of variation ($CV_{wi} = 100\% \times (SD_{wi}/\bar{x}_{wi})$) (Bee et al., 2010). By averaging all within-individual CVs, we obtained the mean within-individual CV (CV_{wi}). This measure allows us to assess the degree of stereotypy of each call property: call traits with a mean CV_{wi} less than 5% were considered static; those with a mean CV_{wi} greater than 12% were considered dynamic; and those with a mean CV_{wi} between 5% and 12% were classified as intermediate (Gerhardt, 1991). In addition, we calculated within-population CVs based on the mean (\bar{x}_{wp}) and standard deviation (SD_{wp}) over all call-recorded individuals in each population ($CV_{wp} = 100\% \times (SD_{wp}/\bar{x}_{wp})$). By averaging all within-population CVs, we obtained the mean within-population CV (CV_{wp}) (Pröhl et al., 2007). Overall CVs (CV_o) were obtained from the grand mean and standard deviation based on averaging all the individual means from our sample ($CV_o = 100\% \times (\text{grand SD}/\text{grand } \bar{x})$). This calculation has also been regarded as among-individual (Bee et al., 2010) and among-population coefficients of variation (Pröhl et al., 2007). The Unini locality was excluded from the analysis of geographic variation because of the small sample size. Based on these measurements, we determined the ratio between the overall and mean within-population variation as CV_o/CV_{wp} .

To compare the difference in calls (with temperature-adjusted traits) between sides of the Amazon River and among populations, nested analyses of variance (ANOVA, populations nested on river sides) were conducted. We used two different models for each call trait: (a) a nested ANOVA without co-factor and (b) a nested anova with size as co-factor, thus constituting a nested ANCOVA. These two approaches were used in order to compare the F statistics obtained through the inclusion of body size as a co-factor in the analyses: if an interaction occurs between co-factors and populations (sampling sites), we would expect a decline in F statistics due to worsening of the model. All statistical analyses, unless otherwise stated, were conducted in SYSTAT 12.0 (Wilkinson, 2007).

We tested the correlation between acoustic/body size distances among sampling sites and respective linear geographic distances by applying Mantel tests. In addition, partial Mantel tests were conducted to check for correlations between acoustic/body size distances and their division by the Amazon River channel, while controlling for effects of linear geographic distance among populations. For this, we constructed a binary correspondence matrix, designating the values '0' and '1' for localities within the same and

between opposite sides of the Amazon River, respectively. Acoustic distance matrices were obtained from the dataset of temperature-adjusted call measurements by calculating pairwise Euclidean distances between their scores on first and second components (which together explained 79.0% of the total acoustic variation) produced by a principal components analysis (PCA). As the scores of the first principal component were correlated with body size (linear regression $r^2 = 0.51$, $F = 9.39$, $p = 0.017$), we regressed them against corresponding mean SVL measurements for each population, and used the residuals as new, size-independent acoustic variables from which new Euclidean distances were calculated. Mantel tests were conducted in ZT (Bonnet & van de Peer, 2002) using permutation of the null models (Anderson & Legendre, 1999), and applying 10 000 randomisations.

3. Results

3.1. *Effect of temperature and body size on acoustic traits*

There was a high temperature dependency of all temporal traits related to the notes of the advertisement call. This relation was not observed in temporal call traits related to the entire call, nor in spectral characters (Table 1). Males produced shorter and more closely spaced notes with increasing temperature. Environmental temperature affected one spectral property (note modulation), which was highly correlated with the temporal trait of note duration ($r_s = 0.53$, $p < 0.001$, $N = 123$). The size of the frogs was related only to spectral parameters of the advertisement call. There were significant negative relationships between body size and the maximum, lowest and highest frequencies. Male SVL explained between 30 and 35% of the variation in spectral traits (Table 1).

3.2. *Stereotypy of acoustic traits*

According to the within-male coefficients of variation observed, the most dynamic traits were related to temporal characteristics of the call. Call duration (range = 0.75–8.73 s) and inter-call interval (range = 1.02–5.51 s) showed the highest variability between individuals and populations. Temporal traits of the notes showed lower coefficients of variation, and spectral measurements were highly stereotyped (Table 2, Figure 3). The lowest and highest frequencies of the call were the most static properties. Overall (or among-population) and within-population coefficients of variation were similar, in-

Table 1.

The effect of temperature and body size on call traits in *Allobates paleovarzensis* ($N = 123$ males).

Trait	Temperature			Body size		
	r^2	F	p	r^2	F	p
NR	0.27	45.14	<0.001*	0.00	0.87	0.353
ND	0.18	28.39	<0.001*	0.00	0.05	0.818
InI	0.21	33.08	<0.001*	0.00	1.56	0.213
CR	0.00	0.09	0.768	0.00	0.61	0.437
CD	0.00	0.91	0.341	0.02	4.11	0.045
IcI	0.00	0.00	0.981	0.00	0.19	0.664
MF	0.00	0.35	0.554	0.30	54.33	<0.001*
LF	0.00	1.13	0.289	0.35	66.55	<0.001*
HF	0.00	1.44	0.232	0.34	63.74	<0.001*
NM	0.11	15.99	<0.001*	0.01	2.47	0.118

Linear regression coefficients of determination (r^2), F statistics and significance values (p) are presented. For body size (SVL), call traits were temperature-adjusted. Significant p values after Bonferroni adjustment are marked with asterisks.

dicating a comparable magnitude in the variability of call traits regardless of grouping (Table 2).

3.3. Geographic variability in acoustic traits

Call properties differed between the sampling localities, although measurements overlapped (Figure 4). Seven of the ten temperature-adjusted call traits differed among populations, and five call traits differed between sides of the Amazon River (Table 3). However, male body size also differed among the sampling sites (Nested ANOVA $F = 3.68$; $p = 0.001$) and between river sides (Nested ANOVA $F = 4.23$; $p = 0.042$). Therefore, analyses of variance were conducted with a new model, which included body size as co-variate (nested ANCOVA). As a result, we still found inter-population and between-river differences in most of the call traits analysed. The differences were distributed among both temporal (related to the notes and calls) and frequency traits (Table 3). The inclusion of body size as a co-variate resulted in generally lower F statistics for the proposed models, indicating that interactive effects between morphology and geography (sampling site) take place in the system.

Table 2.
Descriptive statistics of call traits based on overall acoustic parameter means calculated from mean values of 123 males recorded from 10 localities throughout the range of *Allobates paleovarzensis*, representing natural variation between 23.1 and 28.0°C air temperature.

Trait	Mean ± SD	Range	CV _{wi}	CV _{wp}	CV _o	CV _o /CV _{wp}	Type
NR	5.98 ± 1.37	3.56–10.14	11.1 ± 6.4	17.1 ± 4.2	19.7	1.15	Intermediate
ND	0.04 ± 0.005	0.02–0.06	6.8 ± 3.6	10.5 ± 2.6	11.3	1.08	Intermediate
InI	0.14 ± 0.04	0.07–0.29	16.0 ± 10.2	21.7 ± 3.8	26.3	1.21	Dynamic
CR	0.27 ± 0.07	0.12–0.44	25.0 ± 14.0	23.8 ± 6.6	26.7	1.12	Dynamic
CD	2.31 ± 1.03	0.75–8.73	31.8 ± 13.5	33.2 ± 8.7	44.8	1.35	Dynamic
IcI	2.04 ± 0.75	1.02–5.51	28.1 ± 19.3	29.8 ± 9.7	34.3	1.15	Dynamic
MF	4534.15 ± 192.47	4045.17–5092.62	1.2 ± 1.1	3.6 ± 0.7	4.1	1.12	Static
LF	4147.06 ± 160.87	3645.14–4545.05	0.9 ± 0.7	3.1 ± 0.7	3.7	1.19	Static
HF	4832.32 ± 187.83	4255.77–5316.92	1.0 ± 0.6	3.1 ± 0.6	3.6	1.16	Static
NM	685.26 ± 104.98	462.00–968.51	6.5 ± 3.4	13.4 ± 2.4	14.3	1.07	Intermediate

Mean within-individual, mean within-population and overall coefficients of variation (CV_{wi}, CV_{wp}, CV_o, %) of 10 traits of the advertisement call (adjusted to 25.4°C using linear regression) of *Allobates paleovarzensis*. Traits are classified by type based on mean within-individual CVs according to Gerhardt (1991).

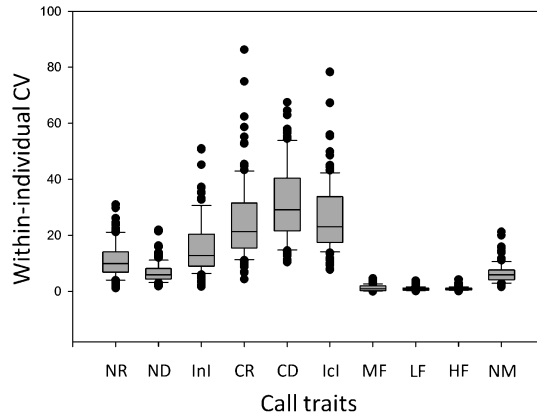


Figure 3. Within-individual coefficients of variation (CV_{wi}) for 10 acoustic properties of the advertisement call of *Allobates paleovarzensis*. Grey boxes indicate the middle 50% of the data (interquartile range), and horizontal lines represent the median values. Dots represent outlying values. Vertical lines indicate the 10th and 90th percentiles.

Body-size differences were correlated with linear geographic distances (Mantel test $r = 0.669$, $p = 0.020$) and not with the side of the Amazon River (Partial Mantel test: $r = -0.128$, $p = 0.289$). However, acoustic differences, after temperature and body size adjustments, did not show correlations with linear geographic distances between population pairs (Mantel test $r = -0.029$, $p = 0.653$) and river sides (Partial Mantel test $r = -0.089$, $p = 0.449$).

4. Discussion

Variability in acoustic sexual signal traits throughout the range of the Amazonian frog *Allobates paleovarzensis* showed comparable magnitudes within and among populations, and was independent of the degree of stereotypy of the acoustic characters. The relative importance of a given call trait as a potential cue for discrimination can be assessed by comparing the ratios between among- and within-population coefficients of variation (Pröhl et al., 2007). Higher between- than within-population CV ratios were found in the polymorphic *Oophaga pumilio*, a dendrobatoid species studied on a similar geographic scale (Pröhl et al., 2007). In a study comprising most of Brazilian Amazonia, the neotropical hyliid frog *Dendropsophus leucophyllatus* also showed a much lower within- than between-population call variability, although this taxon may consist of a complex of cryptic species (Lougheed et

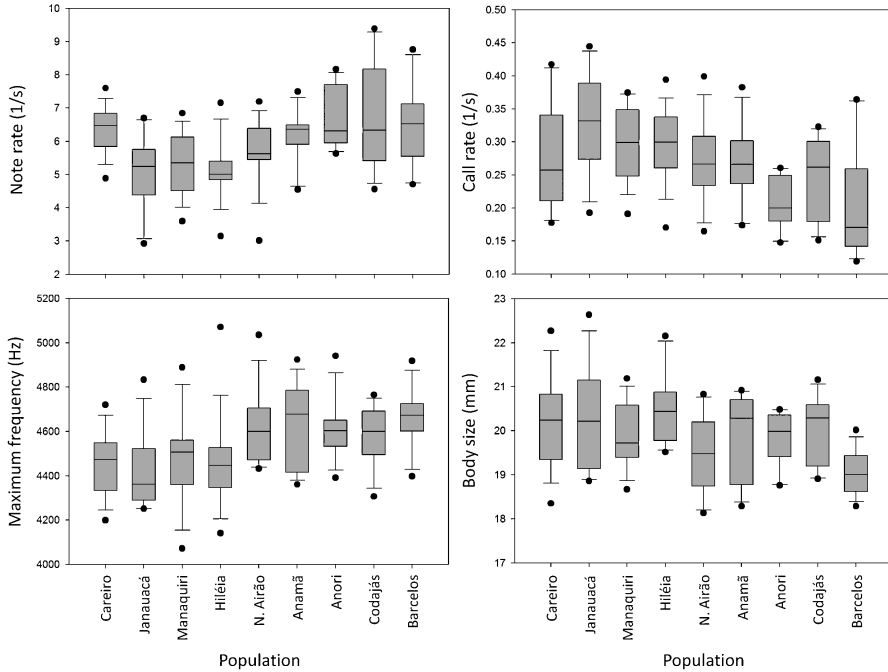


Figure 4. Variation in call traits (adjusted to 25.4°C using linear regression) and body size across the range of *Allobates paleovarzensis* in Central Amazonia. Grey boxes indicate the middle 50% of the data (interquartile range), and horizontal lines represent the median values. Dots represent outlying values. Vertical lines indicate the 10th and 90th percentiles.

al., 2006). Therefore, our findings suggest that none of the call traits analysed is indicated as a potential cue for social recognition or sexual selection between populations of *A. paleovarzensis*.

Ecological and behavioural traits characteristic of the genus may be related to the unexpectedly low overall-/within-population CV ratios observed in *Allobates paleovarzensis*. Here, we hypothesise three non-exclusive causes: (1) Absence of local selective forces causing signal differentiation. Its presence is expected in scenarios involving acoustic character displacement (Lemmon, 2009). At all the localities visited in this study, no other species of the ecologically similar *A. trilineatus* species group was found to occur with *A. paleovarzensis* (Kaefer, unpubl. data). Therefore, we believe that no ecological interspecific selective pressures would be causing important call differentiation (i.e., higher than between-populations) through the range of the species studied. However, it is important to note that the compo-

Table 3.

Nested analysis of (co-)variance of call traits (dependent variables) of *Allobates paleovarzensis*.

Trait	Nested ANOVA				Nested ANCOVA (body size as co-variate)			
	F_{Pop}	p	F_{River}	p	F_{Pop}	p	F_{River}	p
NR	5.21	<0.001*	8.57	0.004*	5.04	<0.001*	8.09	0.005
ND	2.75	0.011	0.38	0.534	2.69	0.012	0.31	0.576
InI	7.41	<0.001*	2.86	0.093	7.09	<0.001*	2.68	0.104
CR	3.66	0.001*	16.79	<0.001*	3.75	0.001*	17.61	<0.001*
CD	3.34	0.002*	0.00	0.966	3.23	0.003*	0.10	0.747
IcI	1.48	0.182	1.20	0.275	1.53	0.164	0.88	0.348
MF	1.96	0.066	19.20	<0.001*	0.62	0.731	14.30	<0.001*
LF	3.40	0.002*	27.70	<0.001*	1.65	0.126	22.77	<0.001*
HF	3.70	0.001*	16.80	<0.001*	2.52	0.019	11.97	<0.001*
NM	3.17	0.004*	0.37	0.540	3.20	0.003*	0.78	0.37

Population (nested in river side) and river side are the dependent categorical variables. In the nested ANCOVA, size is used as the co-variate. Significant p values after Bonferroni adjustment are marked with asterisks.

sition of acoustically coactive sympatric frog species was not important for intraspecific call differentiation in the congener *A. femoralis* (Amézquita et al., 2006). (2) High call trait variability among males. Studies on *A. femoralis* suggest that between-male call variation is pronounced and sufficient to allow statistical distinction of individual males (Gasser et al., 2009). This individual signature in call traits could also be present in *A. paleovarzensis* and lead to the high within-population levels of call variability observed. (3) The use of visual cues to recognise and select mates. Interpopulation variation in calls may have less importance in systems where visual cues are employed in mating interactions. It is likely that *A. femoralis* uses visual components in mate recognition and choice (Luna et al., 2010; Montanarin et al., 2011), as was observed in the dendrobatoid *Oophaga pumilio* (Maan & Cummings, 2009). In addition, the observation of multimodal communication (with the visual component as the secondary channel) during agonistic interactions in the genus lends further support to this hypothesis (Narins et al., 2003, 2005). Therefore, future investigation involving playback experiments on intraspecific mate recognition in *A. paleovarzensis* should provide

us with elements to disentangle the candidate causes for the observed pattern of similar within- and among-population call variability.

Only spectral properties of the advertisement call of *Allobates paleovarzensis* were significantly affected by male body size. This effect is often observed in frogs, and is due to the mass and resonance of the laryngeal sound-producing structures (McClelland et al., 1996), although some species also show temporal properties influenced by male size (e.g., Zweifel, 1968; Castellano et al., 2002; Gasser et al., 2009). The high coefficients of determination observed indicate that all static acoustic properties of the call are under strong morphological constraint, and also that these call properties may be honest indicators of their control factors. Therefore, we should expect that females might impose a stabilising selection on spectral traits of *A. paleovarzensis*, although we cannot disentangle the selective forces driving body size and spectral features in order to evaluate their relative importance in call evolution.

As a neuromuscular mechanism, the regulation of the temporal properties of calls for anurans and other ectotherms is affected by environmental temperature (Prestwich, 1994). In *Allobates paleovarzensis*, all temporal traits related to individual notes, but none related to calls, were affected with this co-factor. This apparently lower sensitivity of calls to temperature may be related to the lower stereotypy (i.e., higher coefficients of variation) of call parameters in relation to those of the notes, making them subject to higher ‘confounding’ intrinsic variability. In fact, Gerhardt & Huber (2002, p. 15) reported a trend for shorter components of the call to be more static, and our data support this observation. Additionally, the social environment composed by co- and heterospecific acoustic signals at the time of the recording may constitute a third source of variation that may affect call traits (Wells & Schwartz, 2007). Therefore, given that call characters were not related to morphological or temperature constraints, it seems likely that, in addition to intrinsic factors, their display may be subject to social regulation.

Both spectral and temporal call traits varied among populations and between sides of the Amazon River. Intraspecific geographic variation in acoustic signals is frequently observed in Amazonian frogs, and highlights the neglected within-species diversity of frogs in the biome, which is also home to a large number of undescribed species (Fouquet et al., 2007; Angulo & Icochea, 2010). Differentiation in Amazonian anurans has been tested

regarding river barrier effects, and provided mixed results associated with geological, idiosyncratic river characteristics (e.g., Gascon et al., 1996, 1998; Loughheed et al., 1999; Funk et al., 2007; Simões et al., 2008; Tsuji-Nishikido et al., 2012). Among these characteristics, width has been indicated as determining the effectiveness of a river barrier in promoting speciation in Amazonian birds (Hayes & Sewlal, 2004) and mammals (Patton & da Silva, 1998). We are not aware of any other study investigating the Amazon, the largest river of the biome, as a promoter of phenotypic differentiation in frogs. Not all acoustic properties of *A. paleovarzensis* showed congruent patterns of divergence, and both river and geographic distance effects were not significant in the overall acoustic variability when summarising all call traits in principal components. The Amazon River was found to be most effective as a dispersal barrier for forest birds along its lower, wider portion, between its confluence with the Negro River and its delta, where it meets the Atlantic Ocean (Hayes & Sewlal, 2004). According to this proposal, our study area lies in a portion where the Amazon River is expected to exert intermediate effects as a vicariant barrier. Future studies should be designed to test the effect of the river on intraspecific differentiation along different portions of its course. In relation to isolation-by-distance effects, a correlation between geographic and acoustic distances would be indicative of neutral processes acting in phenotypic differentiation. Therefore, our results indicate that local stabilising selective forces may be important in call divergence.

There was a positive relation between body size and geographic distances among populations, and as expected, spectral variables of the call followed a similar pattern of variation. However, the different call properties showed divergent patterns of geographic variability through the range of the species, as also observed for the dendrobatoid *Oophaga pumilio* in Costa Rica and Panama (Pröhl et al., 2007). In addition, we could not associate the divergent geographic patterns of call variation to the trait's degree of stereotypy, as observed for green toads from Central Asia (Castellano et al., 2000), which suggests that call variation in some anuran species may not follow this pattern.

Acknowledgements

We are grateful to Ana C. Mello, Anelise Montanarin, Arthur O. Nascimento, Moisés S. Melo, Priscila P. Correa and Raimundo N. Amorim for fieldwork assistance; to Anderson S. Bueno, Luciana K. Erdtmann and Pe-

dro I. Simões for valuable suggestions during this study; to Janet W. Reid for the English revision; and to two anonymous reviewers whose observations greatly improved this paper. This work is part of the PhD thesis of I.L.K. We also thank the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico for financial support (CNPq-CT Amazônia 575572/2008-6). CNPq provided a research fellowship to I.L.K. and a productivity grant to A.P.L. This study was allowed by RAN-ICMBio/IBAMA (licences 13777-2, 18516-1 and 20065-2).

References

- Amézquita, A., Hödl, W., Lima, A.P., Castellanos, L., Erdtmann, L. & Araújo, M.C. (2006). Masking interference and the evolution of the acoustic communication system in the Amazonian dendrobatid frog *Allobates femoralis*. — *Evolution* 60: 1874-1887.
- Amézquita, A., Lima, A.P., Jehle, R., Castellanos, L., Ramos, O., Crawford, A.J., Gasser, H. & Hödl, W. (2009). Calls, colours, shape, and genes: a multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. — *Biol. J. Linn. Soc.* 98: 826-838.
- Anderson, M.J. & Legendre, P. (1999). An empirical comparison of permutation methods for tests of partial regression coefficients in a linear model. — *J. Stat. Comp. Simul.* 62: 271-303.
- Angulo, A. & Icochea, J. (2010). Cryptic species complexes, widespread species and conservation: lessons from Amazonian frogs of the *Leptodactylus marmoratus* group (Anura: Leptodactylidae). — *Syst. Biodiv.* 8: 357-370.
- Bee, M.A., Cook, J.M., Love, E.K., O'Bryan, L.R., Pettitt, B.A., Schrode, K. & Vélez, A. (2010). Assessing acoustic signal variability and the potential for sexual selection and social recognition in Boreal Chorus Frogs (*Pseudacris maculata*). — *Ethology* 116: 564-576.
- Behr, O., von Helversen, O., Heckel, G., Nagy, M., Voigt, C.C. & Mayer, F. (2007). Territorial songs indicate male quality in the sac-winged bat *Saccopteryx bilineata* (Chiroptera, Emballonuridae). — *Behav. Ecol.* 17: 810-817.
- Bonnet, E. & Van de Peer, Y. (2002). ZT: a software tool for simple and partial Mantel tests. — *J. Stat. Soft.* 7: 1-12.
- Boul, K.E., Funk, W.C., Darst, C.R., Cannatella, D.C. & Ryan, M.J. (2007). Sexual selection drives speciation in an Amazonian frog. — *Proc. Roy. Soc. Lond. B: Biol.* 274: 399-406.
- Castellano, S., Cuatto, B., Rinella, R., Rosso, A. & Giacoma, C. (2002). The advertisement call of the European treefrogs (*Hyla arborea*): a multilevel study of variation. — *Ethology* 108: 75-89.
- Castellano, S. & Giacoma, C. (1998). Stabilizing and directional female choice for male calls in the European green toads. — *Anim. Behav.* 56: 275-287.
- Castellano, S., Giacoma, C. & Dujsebayaeva, T. (2000). Morphometric and advertisement call geographic variation in polyploid green toads. — *Biol. J. Linn. Soc.* 70: 341-360.

- Chakraborty, M., Mangiamele, L.A. & Burmeister, S.S. (2010). Neural activity patterns in response to interspecific and intraspecific variation in mating calls in the Túngara Frog. — PLoS One 5: e12898.
- Charif, R.A., Waack, A.M. & Strickman, L.M. (2008). Raven Pro 1.3 user's manual. — Cornell Laboratory of Ornithology, Ithaca, NY.
- Fischer, J., Kitchen, D.M., Seyfarth, R.M. & Cheney, D.L. (2004). Baboon loud calls advertise male quality: acoustic features and their relation to rank, age, and exhaustion. — Behav. Ecol. Sociobiol. 56: 140–148.
- Fouquet, A., Gilles, A., Vences, M., Marty, C., Blanc, M. & Gemmell, N.J. (2007). Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. — PLoS One 32: e1109.
- Funk, W.C., Caldwell, J.P., Peden, C.E., Padial, J.M., De la Riva, I. & Cannatella, D.C. (2007). Tests of biogeographic hypotheses for diversification in the Amazonian Forest Frog, *Physalaemus petersi*. — Mol. Phyl. Evol. 44: 825–837.
- Gascon, C., Loughheed, S.C. & Bogart, J.P. (1996). Genetic and morphological variation in *Vanzolinius discodactylus*: a test of the river hypothesis of speciation. — Biotropica 28: 376–387.
- Gascon, C., Loughheed, S.C. & Bogart, J.P. (1998). Patterns of genetic population differentiation in four species of Amazonian frogs. A test of the Riverine Barrier Hypothesis. — Biotropica 30: 104–119.
- Gasser, H., Amézquita, A. & Hödl, W. (2009). Who is calling? Intraspecific call variation in the arobobatid frog *Allobates femoralis*. — Ethology 115: 596–607.
- Gerhardt, H.C. (1991). Female mate choice in treefrogs: static and dynamic acoustic criteria. — Anim. Behav. 42: 615–635.
- Gerhardt, H.C. & Huber, F. (2002). Acoustic communication in insects and anurans: common problems and diverse solutions. — The University of Chicago Press, Chicago, IL.
- Gomez, D., Richardson, C., Théry, M., Lengagne, T., Léna, J., Plénet, S. & Joly, P. (2011). Multimodal signals in male European treefrog (*Hyla arborea*) and the influence of population isolation on signal expression. — Biol. J. Linn. Soc. 103: 633–647.
- Guerra, M.A. & Ron, S. (2008). Mate choice and courtship signal differentiation promotes speciation in an Amazonian frog. — Behav. Ecol. 19: 1128–1135.
- Hayes, F.E. & Sewlal, J.N. (2004). The Amazon River as a dispersal barrier to passerine birds: effects of river width, habitat and taxonomy. — J. Biogeogr. 31: 1809–1818.
- Heyer, R.W. & Reid, Y.R. (2003). Does advertisement call variation coincide with genetic variation in the genetically diverse frog taxon currently known as *Leptodactylus fuscus* (Amphibia: Leptodactylidae)? — An. Acad. Bras. Ciênc. 75: 39–54.
- Lemmon, E.M. (2009). Diversification of conspecific signals in sympatry: geographic overlap drives multidimensional reproductive character displacement in frogs. — Evolution 63: 1155–1170.
- Lima, A.P., Caldwell, J.P., Biavati, G. & Montanarin, A. (2010). A new species of *Allobates* (Anura: Aromobatidae) from paleovárzea forest in Amazonas, Brazil. — Zootaxa 2337: 1–17.

- Lougheed, S.C., Austin, J.D., Bogart, J.P., Boag, P.T. & Chek, A.A. (2006). Multi-character perspectives on the evolution of intraspecific differentiation in a neotropical hyliid frog. — *BMC Evol. Biol.* 2006: 6-23.
- Lougheed, S.C., Gascon, C., Jones, D.A., Bogart, J.P. & Boag, P.T. (1999). Ridges and rivers: a test of competing hypotheses of Amazonian diversification using a dart-poison frog, *Epipedobates femoralis*. — *Proc. Roy. Soc. Lond. B: Biol.* 266: 1829-1835.
- Luna, A.G., Hödl, W. & Amézquita, A. (2010). Colour, size and movement as visual subcomponents in multimodal communication by the frog *Allobates femoralis*. — *Anim. Behav.* 79: 739-745.
- Maan, M.E. & Cummings, M.E. (2009). Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog. — *Proc. Natl. Acad. Sci. USA* 106: 19072-19077.
- McClelland, B.E., Wilczynski, W. & Ryan, M.J. (1996). Correlations between call characteristics and morphology in male cricket frogs (*Acris crepitans*). — *J. Exp. Biol.* 199: 1907-1919.
- Melendez, K.V. & Feng, A.S. (2010). Communication calls of little brown bats display individual-specific characteristics. — *J. Acoust. Soc. Am.* 128: 919-923.
- Montanarin, A., Kaefer, I.L. & Lima, A.P. (2011). Courtship and mating behaviour of the brilliant-thighed frog *Allobates femoralis* from Central Amazonia: implications for the study of a species complex. — *Ethol. Ecol. Evol.* 23: 141-150.
- Morales, V.R. (2002 “2000”). Sistemática y biogeografía del grupo *trilineatus* (Amphibia, Anura, Dendrobatidae, *Colostethus*), con descripción de once nuevas especies. — *Publ. Asoc. Amigos Doñana* 13: 1-59.
- Narins, P.M., Grabul, D.S., Soma, K.K., Gaucher, P. & Hödl, W. (2005). Cross-modal integration in a dart-poison frog. — *Proc. Natl. Acad. Sci. USA* 102: 2425-2429.
- Narins, P.M., Hödl, W. & Grabul, D.S. (2003). Bimodal signal requisite for agonistic behavior in a dart poison frog, *Epipedobates femoralis*. — *Proc. Natl. Acad. Sci. USA* 100: 577-580.
- Panhuis, T.M., Butlin, R., Zuk, M. & Tregenza, T. (2001). Sexual selection and speciation. — *Trends Ecol. Evol.* 16: 364-371.
- Patton, J.L. & da Silva, M.N.F. (1998). Rivers, refuges, and ridges: the geography of speciation of Amazonian mammals. — In: *Endless forms: species and speciation* (Howard, D.J. & Berlocher, S.H., eds). Oxford University Press, Oxford, p. 202-213.
- Prestwich, K.N. (1994). The energetics of acoustic signaling in anurans and insects. — *Am. Zool.* 34: 625-643.
- Pröhl, H., Hagemann, S., Karsch, J. & Höbel, G. (2007). Geographic variation in male sexual signals in Strawberry Poison Frogs (*Dendrobates pumilio*). — *Ethology* 113: 825-837.
- Rodríguez, A., Nuez, D. & Alonso, R. (2010). Intraspecific variation in the advertisement call of the Cloud-Forest Frog *Eleutherodactylus glamyrus* (Anura: Eleutherodactylidae). — *J. Herpetol.* 44: 457-466.
- Ryan, M.J., Cocroft, R.B. & Wilczynski, W. (1990). The role of environmental selection in intraspecific divergence of mate recognition signals in the cricket frog, *Acris crepitans*. — *Evolution* 44: 1869-1872.

- Ryan, M.J., Rand, A.S. & Weigt, L.A. (1996). Allozyme and advertisement call variation in the tungara frog, *Physalaemus pustulosus*. — Evolution 50: 2435–2453.
- Ryan, M.J. & Wilczynski, W. (1991). Evolution of intraspecific variation in the advertisement call of a cricket frog (*Acris crepitans*, Hylidae). — Biol. J. Linn. Soc. 44: 249–271.
- Simões, P.I., Lima, A.P., Magnusson, W.E., Hödl, W. & Amézquita, A. (2008). Acoustic and morphological differentiation in the frog *Allobates femoralis*: relationships with the upper Madeira River and other potential geological barriers. — Biotropica 40: 607–614.
- Smith, M.J. & Hunter, D. (2005). Temporal and geographic variation in the advertisement call of the Booroolong Frog (*Litoria booroolongensis*: Anura: Hylidae). — Ethology 111: 1103–1115.
- Tsuji-Nishikido, B.M., Kaefer, I.L., Freitas, F.C., Menin, M. & Lima, A.P. (2012). Significant but not diagnostic: differentiation through morphology and calls in the Amazonian frogs *Allobates nidicola* and *A. masniger*. — Herpetol. J.: in press.
- Vences, M. & Wake, D.B. (2007). Speciation, species boundaries and phylogeography of amphibians. — In: Amphibian biology, systematics (Heatwole, H. & Tyler, M.J., eds). Surrey Beatty & Sons, Chipping Norton, p. 2613–2671.
- Verburgt, L., Ferreira, M. & Ferguson, J.W.H. (2011). Male field cricket song reflects age, allowing females to prefer young males. — Anim. Behav. 81: 19–29.
- Wells, K.D. & Schwartz, J.J. (2007). The behavioral ecology of anuran communication. — In: Hearing and sound communication in amphibians (Narins, P.M., Feng, A.S., Fay, R.R. & Popper, A.N., eds). Springer-Verlag, New York, NY, p. 44–86.
- Wilkinson, L. (2007). SYSTAT: the system for statistics. — SYSTAT, Evanston, IL.
- Zweifel, R.G. (1968). Effects of temperature, body size, and hybridization on mating calls of toads, *Bufo a. americanus* and *Bufo woodhousii fowleri*. — Copeia: 269–285.