

Intra-specific variation in advertisement call of *Odontophrynus cordobae* (Anura, Cycloramphidae): a multilevel and multifactor analysis

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Abstract. Advertisement call is a complex signal that can vary at different scales and the characterization of this variation is crucial to understand its adaptive function. In our study, we examined different factors that could influence intra and inter-population call variation in *Odontophrynus cordobae*. We analyzed calls of individuals from different breeding sites across the species distribution, including a syntopic site with their related species *O. americanus*. Dominant frequency, pulse rate, call duration and pulses/call were the acoustic parameters that showed the lowest intra-individual variation ($CV \leq 3\%$). The discriminant function analysis (DFA) assigned 92% of calls to the correct individual, indicating that the advertisement calls of *O. cordobae* are individually distinctive. Univariate and multivariate statistical analysis showed a significant contribution of pulse rate in differentiation of populations. Interpulse interval and pulse rate showed a moderate clinal variation, even when syntopic populations were excluded from the analysis. We found no association between acoustic and linear geographical or altitudinal distances and observed an evident divergence between the syntopic population and the other populations analyzed. This result is reinforced by DFA, which showed a differentiation among syntopic and allopatric populations of *O. cordobae*. We discuss our results from the hypothesis of a possible character displacement influencing call properties in this species in syntopy with their related species *O. americanus*. However, new syntopic areas and tests of female preferences would be needed to strengthen our results.

Keywords: anuran communication, bioacoustics, geographic variation, individual differences.

Introduction

The sexual communication system of animals is crucial for successful reproduction: it facilitates mate detection, serves as an indicator of identity, and can indicate mate quality among conspecifics (Pröhl et al., 2007). In animals, signals vary at multiple scales: within and among species, among populations, among individuals and even for an individual. Intra-specific variation in communication signals and receivers has proven to be crucial for the understanding of mating signals evolution in anurans, because it is the raw material for selection to act on (Loftus-Hills and Littlejohn, 1992). Acoustic properties of anuran signals could be

placed along a continuum from 'static' to 'dynamic' based on patterns of within-male variability (Gerhardt, 1991). Moreover, call properties at the static and dynamic ends of the continuum are often under different selection regimes (Bee et al., 2010). Several studies have examined the function of the different type of properties (Gerhardt, Dyson and Tanner, 1996; Castellano and Giacoma, 1998; Baugh and Ryan, 2009, 2010). Properties under stabilizing preferences are more stereotyped than those under directional preferences (Gerhardt, 1991). Furthermore, there is evidence that static and dynamic properties show different patterns of geographic variation (Giacoma and Castellano, 2001). Consequently, the analysis of signal variation at different levels of organization might provide an important source of information for the understanding of signal evolution (Castellano et al., 2002b).

The genus *Odontophrynus* is endemic to South America and currently consists of 10 recognized species distributed in southern and

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eastern parts of the continent (Frost, 2011). Based on external morphology, *Odontophrynus* species are clustered in three groups: *americanus*, *cultripes* and *occidentalis*. The *americanus* group is currently represented by four species: *O. americanus*, *O. lavillai*, *O. cordobae* and *O. maisuma*. Distribution of *Odontophrynus cordobae* (formerly *O. americanus* diploid form) is restricted to central and north-western Córdoba and some populations in the south of Santiago del Estero province, Argentina (Martino and Sinsch, 2002; Rosset et al., 2006). The advertisement call of *O. cordobae* is characterized by a long single pulse train or pulse group (Martino and Sinsch, 2002). However, intra and inter-population variation in advertisement call has not been studied for this species or other species in the genus. Therefore, our aims were: 1) to examine call variation among and within populations; 2) to study the geographic variation in advertisement calls along the known range of *O. cordobae*; and 3) to evaluate differences between syntopic and non-syntopic populations of this species.

Materials and methods

Call recording and analysis

Advertisement calls were recorded along the distribution of *Odontophrynus cordobae* in 12 populations from central and north-western Córdoba province, Argentina (fig. 1), in the periods 1999–2002 and 2006–2009. The altitudinal range was 550–1450 m.a.s.l. Individuals were acoustically recorded during spring-summer months (September–March), between 8 pm and 5 am. Breeding sites with active male chorus were found in backwaters along streams and in temporary and permanent ponds. We did not collect individuals immediately after the acoustic records; therefore we do not include the body measurements of each male as parameters in the analyses developed in this study. The southernmost population, La Escondida, represents a syntopic site between *O. cordobae* and its cryptic polyploid counterpart, *O. americanus* (Grenat, Salas and Martino, 2009). At present, hybrids between these species are not known. A series of 3–10 advertisement calls per individual of a total of 91 individuals were recorded in the mating sites using a Walkman Digital AudioTape (DAT) Sony™ TCD-100 with stereo microphone ECM-MS907 Sony™ located to 30 cm approximately of focal individual. Immediately after the recording, water temperature (to the nearest 0.1°C) was registered at



Figure 1. Map of the study area indicating the geographical position of *Odontophrynus cordobae* populations sampled in central and north-western Córdoba province.

each individual calling site (range = 13.0–23.2°C). Individuals were not captured, except in La Escondida site. Since this *O. cordobae* population occurs in syntopy with populations of the tetraploid cryptic species, *O. americanus*, a cytogenetic and erythrometric analysis (Grenat et al., 2009) was necessary for ploidy determination of recorded individuals.

Acoustic signals were digitized and analyzed by means of oscillograms, sonograms and power spectra using the software Adobe® Audition™ 1.0 (FFT (*fast Fourier transform*): 1024 points; sampling rate: 44.1 KHz; bit depth: 16 bit). We analyzed calls of all range of series (at the beginning, in the middle and at the end of the recording), following to Castellano et al. (2002b). The figure of the oscillogram and sonogram was performed using Raven Lite 1.0. Each call series was characterized by five parameters: (1) Call Duration (CD) [ms]; (2) Pulses/Call (PC) [N]; (3) Pulse Duration (PD) [ms]; (4) Interpulse Interval (IPI) [ms]; and (5) Dominant Frequency (DF) [Hz]. Pulse Rate (PR) [pulses/s] and Duty Cycle (DC) [Pulse Duration/Interpulse Interval] were also calculated (Martino and Sinsch, 2002).

Statistical analyses

Descriptive statistics were calculated for all call parameters. Linear regressions between call variables and water temper-

ature were performed to assess the effect of this environmental parameter on acoustic traits. Temperature dependent acoustic parameters were adjusted to 19°C, following Heyer and Reid (2003).

Call variation within and among populations. For inter-population call comparison, separate univariate analyses of variance (ANOVA) were performed for each call character. If significant differences were obtained, pairwise Tukey's HSD tests were applied to determine which groups showed differences.

Coefficient of variation ($CV = (SD/Mean) * 100$) were used to compare patterns of variation at different levels: within individual (among calls from the same individual during a single bout of calling), within population (among individuals from the same population) and, among populations. CVs of higher levels were estimated using the mean values of the acoustic parameter obtained at the immediately lower level, applying an approach similar to that employed in the variance computation in a nested ANOVA model (see Castellano and Giacoma, 1998). We tested the dependence of the CVs on the sample size because we did not analyze the same number of calls in each individual. We found similar values using the total number of calls or using randomly selected subsamples of three calls per individual. However, for simplicity and to avoid bias in estimates of variation, we report the CVs calculated based on the same number of calls for individual ($n = 3$).

Discriminant function analysis (DFA) was used to statistically examine the individual and population distinctiveness when all call variables were considered. The discriminant functions generated in a DFA can be used to classify signals as belonging to particular individuals. Furthermore, classification success serves as a measure of how well the linear combinations of variables in the discriminant functions distinguish among individuals (Bee et al., 2001).

Geographic pattern of acoustic variation. The mean population values of temperature-adjusted call variables were regressed against both latitude and longitude to evaluate if there was a clinal call variation.

A discriminant analysis was then performed to examine how calls varied among populations. To evaluate associations between geographic distances and call dissimilarity, we performed Mantel tests using MANTEL for Windows 1.18 (Cavalcanti, 2005). Ten thousand iterations were conducted to assess the significance of the results. The Mantel test calculates correlations among similarity/dissimilarity and distance matrices considering that the same data point is used for multiple comparisons (Bernal, Guarnizo and Lüddecke, 2005). We built a Mahalanobis distance dissimilarity matrix between the means of call variables, and correlated it with a matrix of linear geographical distances between all pairs of study sites.

Acoustic variation between syntopic and non-syntopic populations. Populations were divided into three geographic groups along the *O. cordobae* distribution, according to distance between them and geographic location: 1) northern and central populations (far allopatric); 2) southern populations (near allopatric); and 3) the syntopic population of La Escondida. For acoustic comparisons among

groups we performed separated paired ANOVAs for each call parameter.

Discriminant function analysis (DFA) was used to statistically examine the group distinctiveness when all call variables were considered. Phenetic clustering of groups based on acoustic distance was applied using the unweighted pair-group method with arithmetic averaging (UPGMA).

Results

Calls of all populations showed the same basic structural features: a single pulse group; a spindle shape oscillogram, in which the pulses located at the beginning of the call have a low intensity, followed by increasing intensity pulses, and a decrease in pulse intensity at the end of call (fig. 2). Table 1 shows population means and standard deviations for temperature-adjusted (19°C) acoustic properties of advertisement calls. Temperature was positively associated with pulses/call ($r = 0.37$; $p < 0.001$), dominant frequency ($r = 0.40$; $p < 0.001$) and, pulse rate ($r = 0.86$; $p < 0.0001$); and negatively with call duration ($r = -0.78$; $p < 0.0001$), pulse duration ($r = -0.88$; $p < 0.0001$) and, interpulse duration ($r = -0.77$; $p < 0.0001$). Temperature was not correlated with duty cycle ($r = 0.03$; $p = 0.76$).

Call variation within and among populations

Table 2 shows the mean CVs calculated for each acoustic variable, at the three levels of analysis considered. As expected the CVs of all parameters of call were narrow at individual level. Pulse rate and dominant frequency presented the lowest variation at within individual and within population levels. CVs for call duration, pulse/call, dominant frequency and pulse duration were considerably lower within individuals than within populations. CVs for interpulse interval, pulse rate and duty cycle increased with the level of analysis, from within individual to among populations.

Individual acoustic distinctiveness was examined using discriminant function analysis and including only the five measured call parameters. Discriminant functions were highly signif-

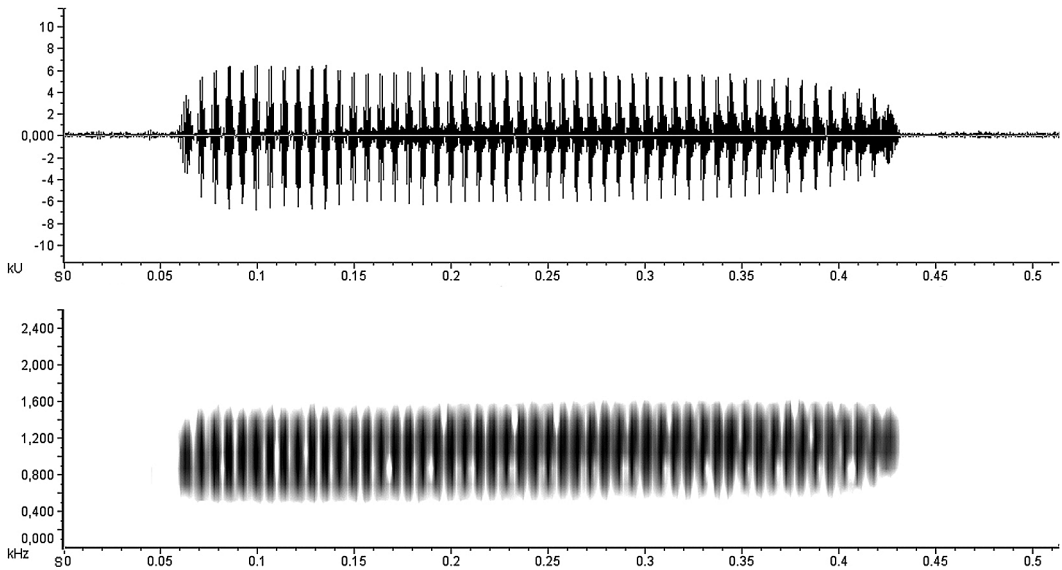


Figure 2. Oscillogram and spectrogram of a representative *Odontophrynus cordobae* advertisement call.

Table 1. Mean and standard deviation of acoustic measurements taken on call series of *O. cordobae* from 12 breeding sites. Parameters abbreviations: CD: call duration; P/C: pulses/call; DF: dominant frequency; PD: pulse duration; IPI: interpulse interval; PR: pulse rate; DC: duty cycle. *N*: number of individual analysed.

Locality	<i>N</i>	Acoustical parameters						
		CD (ms)	PC	PD (ms)	IPI (ms)	DF (Hz)	PR	DC
Tanti	3	446.2 ± 9.4	51.6 ± 2.4	3.0 ± 0.5	5.4 ± 0.2	1096.5 ± 70.7	119.5 ± 4.4	0.55 ± 0.12
Los Gigantes	5	521.3 ± 12.3	59.8 ± 1.6	3.9 ± 0.1	5.1 ± 0.1	1073.7 ± 57.3	113.6 ± 0.3	0.75 ± 0.05
San Clemente	7	498.0 ± 51.9	54.3 ± 4.1	3.3 ± 0.3	5.8 ± 0.4	977.7 ± 71.9	109.7 ± 4.7	0.56 ± 0.08
Villa Las Rosas	3	428.2 ± 8.5	46.1 ± 1.4	5.0 ± 0.3	4.3 ± 0.3	874.1 ± 64.3	114.0 ± 1.5	1.30 ± 0.22
Villa Gral. Belgrano	15	433.7 ± 51.5	48.5 ± 3.4	4.1 ± 0.5	5.0 ± 0.5	987.0 ± 41.6	116.9 ± 4.5	0.85 ± 0.16
Santa Rosa	10	484.5 ± 37.9	49.9 ± 3.0	4.0 ± 0.4	5.8 ± 0.7	1041.4 ± 98.2	102.4 ± 6.1	0.70 ± 0.13
Cañada del Sauce	6	483.7 ± 39.6	51.7 ± 3.5	3.7 ± 0.4	5.8 ± 0.5	1064.3 ± 49.1	107.6 ± 4.5	0.62 ± 0.15
Berrotarán	12	412.1 ± 17.9	49.9 ± 2.4	3.7 ± 0.1	4.4 ± 0.3	933.6 ± 59.1	129.3 ± 6.2	0.87 ± 0.07
Río de los Sauces	7	479.2 ± 31.2	56.9 ± 5.5	3.8 ± 0.4	4.3 ± 0.7	998.3 ± 50.0	121.6 ± 4.4	0.90 ± 0.16
Elena	6	443.4 ± 9.0	54.8 ± 1.6	3.8 ± 0.3	4.6 ± 0.3	970.7 ± 59.2	122.8 ± 3.4	0.85 ± 0.14
A° San Francisco	5	487.3 ± 77.2	54.0 ± 10.6	3.9 ± 0.1	5.4 ± 0.02	1022.6 ± 8.3	111.0 ± 1.9	0.7 ± 0.02
La Escondida	12	410.7 ± 49.9	52.3 ± 4.6	3.7 ± 0.2	3.8 ± 0.3	1007.7 ± 65.0	135.3 ± 9.5	0.98 ± 0.09

Table 2. Coefficients of variation of advertisement call parameters calculated at three different levels of analysis. CD: call duration; P/C: pulses/call; DF: dominant frequency; PD: pulse duration; IPI: interpulse interval; PR: pulse rate; DC: duty cycle.

Analysis level	CD	P/C	DF	PD	IPI	PR	DC
Within individual	2.8 ± 1.7	2.9 ± 1.7	0.9 ± 1.4	4.7 ± 3.0	5.1 ± 3.5	2.5 ± 1.6	8.6 ± 6.6
Within population	7.1 ± 4.6	6.9 ± 4.6	5.9 ± 2.1	8.2 ± 4.0	7.4 ± 4.2	3.8 ± 2.0	13.9 ± 6.3
Among population	8.0	6.6	5.1	8.6	13.9	8.5	20.3

icant (Wilks' lambda < 0.0001; canonical correlation = 0.997; $p < 0.0001$), and the first two functions explained 90% of the individual variation. The first function was strongly associated with call duration and interpulse interval, while the second function was mainly associated with the dominant frequency. DFA correctly classified 92% of the calls within its respective male.

Univariate analysis demonstrated significant differences among populations in all acoustic parameters (CD: $F_{11,80} = 5.04$, $p < 0.0001$; P/C: $F_{11,80} = 4.78$, $p < 0.0001$; DF: $F_{11,80} = 4.71$, $p < 0.0001$; PD: $F_{11,80} = 6.59$, $p < 0.0001$; IPI: $F_{11,80} = 16.51$, $p < 0.0001$; PR: $F_{11,80} = 22.14$, $p < 0.0001$; DC: $F_{11,80} = 14.58$, $p < 0.0001$). Pairwise tests showed that calls from La Escondida population were highly different from the remaining populations in four out of seven acoustic variables (CD, IPI, PR and DC; Tukey test, $p < 0.05$).

When grouping populations according to their acoustic characteristics using the discriminant function analysis, we were able to discriminate individuals from different populations. This analysis yielded seven discriminant functions, the first four of which were highly significant and accounted for 98.3% of the total variation. Although there was some overlapping among populations, when grouping all individuals, 83.1% were correctly assigned to their correct population. Populations of *O. cordobae* were mainly distinguished by function 1 (Wilks' lambda = 0.032; canonical correlation = 0.90; $p < 0.0001$), which was mostly composed of pulse rate (fig. 3).

Geographic pattern of acoustic variation: influence of latitude, longitude, altitude and distance

Simple regressions between call variables and latitude or longitude coordinates showed a north-south and west-east decrease in call duration ($r = 0.29$; $p < 0.01$ and $r = -0.45$; $p < 0.0001$, respectively) and interpulse interval ($r = 0.53$; $p < 0.0001$ and $r = -0.51$; $p < 0.0001$, respectively) and a north-south and

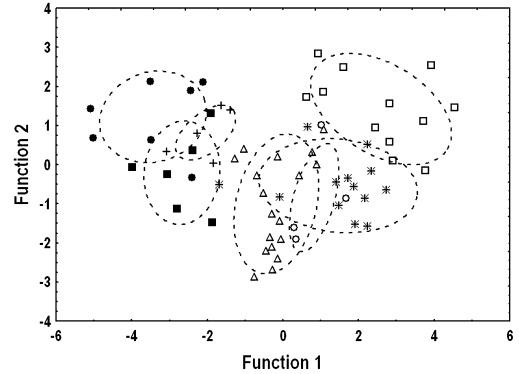


Figure 3. Acoustic differentiation among seven populations of *Odontophrynus cordobae*: Santa Rosa (●), Cañada del Sauce (■), San Clemente (+), Villa General Belgrano (△), Berrotarán (*), Elena (○) and La Escondida (□). Ellipses give the 95% confidence regions for each group.

west-east increase in pulse rate ($r = -0.47$; $p < 0.0001$ and $r = 0.55$; $p < 0.0001$) and duty cycle ($r = -0.53$; $p < 0.0001$ and $r = 0.48$; $p < 0.0001$). Pulse duration and pulse/call did not show a significant correlation with either latitude (PD: $r = -0.15$; $p = 0.19$; PC: $r = 0.005$; $p = 0.96$) or longitude (PD: $r = 0.07$; $p = 0.52$; PC: $r = -0.16$; $p = 0.14$). Dominant frequency was only correlated with longitude ($r = -0.48$; $p < 0.0001$), and decreased eastward.

The Mantel test comparing the geographic distance and call distance matrices showed no significant differences. The Mantel test correlation matrix was $r = 0.106$ and the one-tail probability was $p[\text{random } Z < \text{observed } Z] = 0.748$. Finally, associations between altitudinal and call distances were not significant (Mantel test; $r = -0.093$; $p[\text{random } Z < \text{observed } Z] = 0.36$).

Acoustic variation between syntopic and non-syntopic populations

In the regressions analyses of the different call parameters versus latitude performed excluding the syntopic population, we found different results only for the call duration, which was not associated with latitude ($r = 0.18$; $p = 0.14$)

and, for the dominant frequency, which showed a north-south decrease ($r = 0.27$; $p < 0.05$).

A cluster analysis based on square Mahalanobis distances, including all populations, shows three acoustic groups (fig. 4). These groups, except the localities of Villa General Belgrano and A° San Francisco, largely represent three geographical groups, with the syntopic population well differentiated. Thus, in order to deepen this analysis, populations were divided into three geographic groups: 1) far allopatric: northern and central populations; 2) near allopatric: southern populations; and 3) syntopic population. Univariate analysis (ANOVA) showed acoustic differences between the syntopic population and the other two

groups in: call duration (1-3: $F_{1,45} = 7.69$, $p < 0.01$; 2-3: $F_{1,43} = 6.35$, $p < 0.05$), pulse rate (1-3: $F_{1,45} = 56.73$, $p < 0.0001$; 2-3: $F_{1,43} = 18.45$, $p < 0.0001$), interpulse interval (1-3: $F_{1,45} = 59.46$, $p < 0.0001$; 2-3: $F_{1,43} = 16.89$, $p < 0.0001$) and duty cycle (1-3: $F_{1,45} = 37.92$, $p < 0.0001$; 2-3: $F_{1,43} = 13.20$, $p < 0.001$). Non-syntopic groups differed significantly in pulse rate ($F_{1,66} = 9.46$, $p < 0.01$), interpulse interval ($F_{1,66} = 11.24$, $p < 0.01$) and duty cycle ($F_{1,66} = 13.20$, $p < 0.001$).

To evaluate the magnitude of acoustic differences between geographical groups, we performed a discriminant function analysis (procedure: forward selection). DFA was significant (Wilks' lambda = 0.53; canonical correlation = 0.63; $p < 0.0001$) and only four parameters were maintained in the analysis (CD, DF, IPI and PR). The first canonical function explained 93.3% of the total variation among groups and was positively related to pulse rate (PR standardized coefficient = 0.696), and negatively to interpulse interval (IPI standardized coefficient = -0.558). When pooling all individuals, 62.5% were correctly assigned to their group of origin. The highest number of individuals correctly assigned belonged to the syntopic population (83.3%), while individuals from groups 1 and 2 largely overlapped (fig. 5A). The clus-

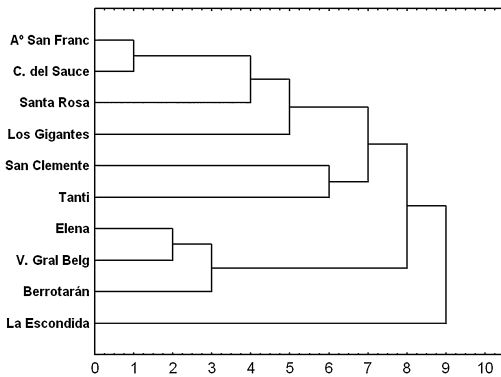


Figure 4. Cluster analysis based on square Mahalanobis distances showing the similar acoustical population groups.

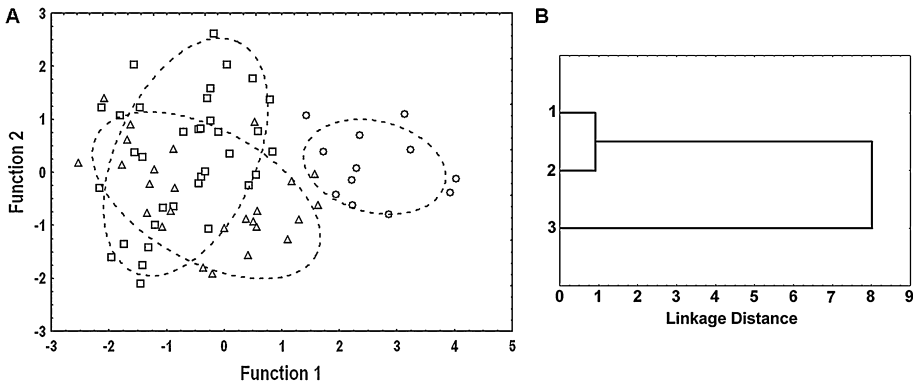


Figure 5. Acoustic distinction among three population groups. (A) Discriminant function analysis: far allopatric (squares, group 1), near allopatric (triangles, group 2) and syntopic (circles, group 3) populations; ellipses give the 95% confidence regions for each group. (B) UPGMA cluster of acoustic distances among the three populations groups; 1: far allopatric, 2: near allopatric, and 3: syntopic populations.

ter analysis using UPGMA method based on squared Mahalanobis distances resulted in a tree reflecting similar results to those of DFA (fig. 5B). The branch of the syntopic population (group 3) differed notably from the other two population groups.

Discussion

Studies of intra-specific geographic variation have two main goals: the description of the spatial patterns of variation, and the analysis of the forces responsible for these patterns (Cracraft, 1989). In this study we examined different factors that can influence intra and inter-population call variation in *Odontophrynus cordobae*.

Call variation within and among populations

Different acoustic properties may be subject to different selective pressures and constraints, and so these may evolve at different rates (Cocroft and Ryan, 1995). Gerhardt (1991) categorized the call properties as static ($CV < 5\%$) and dynamic ($CV > 12\%$) using coefficients of variation. Following this criteria we should classify all measured parameters and the pulse rate of *O. cordobae* advertisement call as static properties. However, static and dynamic properties also differ in other aspects not directly related to differences in variability (Castellano and Giacoma, 1998). Static properties mainly affect the quality of signal, whereas dynamic properties determine the quantity of signalling (Gerhardt, 1991). Dominant frequency, pulse rate, call duration and pulse/call were the call parameters that showed the lowest intra-individual variation ($CV \leq 3\%$). Based on this categorization, we would predict patterns of stabilizing or directional female preferences generally favouring values near the population mean for these acoustic parameters (Bee et al., 2010). According to previous studies on anuran (Gerhardt, 1991; Castellano and Giacoma, 1998) and insects calls (Jang and Gerhardt, 2006), spectral parameter and pulse rate represented static

properties. These static properties are subject to strong constraints, either morphological (spectral parameters) or physiological (pulse rate). The constraints are important in signal evolution because they may indirectly expose signals to the effects of evolutionary forces (Castellano et al., 2002a). Furthermore, from information theory, it is known that the stronger the restrictions on a signal property, the greater the information redundancy that can be transmitted and the lower the risk of transmission errors (Nauta, 1972). If this rule holds in biological systems too, the acoustic properties with greater restrictions should be the most appropriate to transmit those messages, which misinterpretation would have a higher cost in fitness, for both signaller and receiver.

As in other anurans with pulsed advertisement calls, pulse rate was strongly affected by temperature (Sullivan, 1992; Castellano and Giacoma, 1998). Temperature restricts the contractile properties of both trunk and laryngeal muscles involved in the production of the call (Wells and Taigen, 1992). According to Castellano and Giacoma (1998) this effect can explain why, at high temperatures, males can produce calls with higher pulse rates than at low temperatures, but does not explain the low variability observed in pulse rate. These authors suggested that the physiological constraint should not avoid males from calling with pulse rates lower than those reachable at a given temperature range. Gerhardt (1991) proposed that the characteristically narrow range of variation of pulse rates in a population may be maintained by female choice, i.e.: it is an adaptation to female preferences. Under this hypothesis, a male does not need to produce a call with a pulse rate that falls within a narrow range to attract a conspecific female, but the relative mating success of a male producing calls with pulse rates outside the optimum range will almost certainly be reduced (Gerhardt, 1991). For example, females of *Hyla chrysoscelis* more strongly reject alternatives with lower-than-average pulse rates than those with higher-than-average pulse rates; fe-

males of *H. versicolor* show the opposite pattern (Gerhardt and Doherty, 1988).

The number of pulses per call was also a static property and, according to Castellano et al. (2002b), its low variation might arise from the combined effect of the low variation of call duration and the morphology that constrains the vibration frequency of the arytenoid cartilages. Furthermore, this parameter is not expected to change drastically because the pulse duration, interpulse interval and call duration showed little variation.

Typically, relatively long signals exhibit greater intra-individual variability than shorter signals (Bee et al., 2010). The categorization of call duration as a static property departs from the general pattern found in other anurans (Gerhardt, 1991). Since call duration depends upon the activity of muscles that force the air from the lungs through the larynx, the low intra-individual CVs for this parameter in a single calling bout might be caused by motor pattern stereotypy rather than morphological constraints (Castellano et al., 2002b). Call duration of *Hyla cinerea* and *Pseudacris crucifer* was classified as a static property, although the mean CVs of this parameter was notably larger than that of dominant frequency (Oldham and Gerhardt, 1975; Doherty and Gerhardt, 1984). In addition, females of many other frogs commonly exhibit strong directional preferences for longer calls (Ryan and Keddy-Hector, 1992; Gerhardt and Huber, 2002). Consequently, Gerhardt (1991) hypothesized that the different properties of the call may convey different types of information. Pulse rate and fundamental frequency, subject to stabilizing preferences, may be important for species recognition, while call duration may be important for mate choice.

Masking interference may be one of the many selective forces responsible for call differentiation in a breeding season or nightly calling periods. Calls of heterospecific males may produce some masking, but conspecific calls are a much more important source of interference; this interference can affect patterns of female choice

(Gerhardt, 1994b). The discriminant function analysis using the five measurable acoustic variables assigned approximately 92% of advertisement calls to the correct individual, indicating that the advertisement calls of *O. cordobae* are individually distinctive. This result is in agreement with other studies that have also used multivariate statistical analysis to characterize the individual distinctiveness of anuran advertisement calls (Bee and Gerhardt, 2001; Bee et al., 2010). The expected efficacy of selection based on any pattern of preference will be determined primarily by the minimum difference in any acoustic property that obtains the preference relative to the between-male variability in this property in the population (Gerhardt, 1994). Consequently, we found that the parameters most strongly associated to the first two discriminant functions coincided with those parameters that showed the lowest intra-individual variation (Call duration, dominant frequency and interpulse interval associated with pulse rate).

By comparing the variability of the signal at different levels, we have also shown that different properties show different trends. In particular, at the inter-population level, the pulse interval, duty cycle and pulse rate were more variable than at the intra-population level, whereas the remaining properties did not change significantly or were less variable. According to Castellano and Giacoma (1998), these results could mean that the call properties studied, despite being subject to stabilizing preferences, play a more relevant role in the process of geographical differentiation.

We found statistically significant differences among populations in every acoustic parameter. Despite the fact that an important consequence of the anuran advertisement call is species recognition, a substantial variation in call characters among populations is expected (Ryan and Wilczynski, 1991). Multivariate statistical analysis showed a significant contribution of pulse rate in acoustic differentiation of *O. cordobae* populations. The pulse rate has been commonly used for the distinction between related anu-

ran species (e.g., *Pseudacris maculata*/*P. triseriata* – Lemmon et al., 2007), many of them belonging to diploid/polyploid complexes (e.g. *Hyla chrisoscelis*/*H. versicolor* – Ralin, 1977; *Neobatrachus* – Roberts, 1997; *Bufo viridis* complex – Stöck, 1998). Furthermore, pulse rate has been used as a diagnostic character for acoustic differentiation between *O. cordobae* and *O. americanus* (Martino and Sinsch, 2002).

Geographic pattern of acoustic variation

Statistical analysis showed that populations not only differ in some acoustic characters, but also that these differences are not entirely random over geographic space. A moderate latitudinal and longitudinal cline of acoustic variation was found. Interpulse interval and pulse rate were the only variables that showed a clinal variation, even when syntopic populations were excluded from analysis, although in this case their association with latitude was less pronounced. The interpulse interval increased towards allopatric populations, with a consequent decrease in pulse rate. Since pulse duration and pulse/call was not correlated with latitude or longitude, we could expect that a decrease in interpulse interval generates an increase in the same direction on pulse rate and duty cycle. Giacoma and Castellano (1998) suggest that this variability could be attributed to local adaptations, differences in the availability of resources or a reduction in gene flow. The pattern of geographic variation observed in interpulse interval and pulse rate is consistent with the results obtained in the analysis of CVs and with the notion that sexual selection may generate population differences in mate recognition signals (Ryan and Wilczynski, 1991).

We found no correlation between dominant frequency and latitude. The frequency of most anuran advertisement calls is directly related to the frequency at which the vocal cords vibrate, and therefore depends upon their mass and tension (Castellano and Giacoma, 1998). For this reason, in most anurans, the dominant frequency is inversely associated with body

size (Cocroft and Ryan, 1995; Castellano et al., 1999). In some toads and frogs, females prefer lower frequencies resulting in sexual selection for larger males (Ryan and Keddy-Hector, 1992). Unfortunately, our study fails to examine the possible relationship of the acoustic variables and body size because we did not have information about the morphology of the caller in our analysis, since we did not capture them. However, analysis of geographic variation in SVL of *O. cordobae* adult males from localities considered in the present study and other distribution points showed that it was not associated with latitude (Grenat, Salas and Martino, 2012). Thus, the absence of clinal geographic patterns in body length is consistent with the absence of the same patterns in the call frequencies.

We found no association between acoustic and linear geographical distances. This result is consistent with that of the DFA (fig. 3), in which groups of acoustically similar populations were not directly related to the geographic proximity between them.

Acoustic variation between syntopic and allopatric populations

Because the advertisement call is a powerful tool to distinguish among anuran species, it is expected that the acoustic differences will be more pronounced among sympatric populations of species with different ploidy levels than between allopatric populations of those species (Arnetz and Bauer, 1996). Consistent with this, the theory of reproductive characters displacement predicts the accentuation of differences in pre-reproductive behaviour in a sympatry area between two species of common origin (Brown and Wilson, 1956). The divergent character displacement of male courtship behaviours, that serve as isolating pre-reproductive mechanisms, has been documented in a wide variety of organisms (Grant, 1972; Waage, 1979; Howard, 1993), but mostly involving anuran advertisement vocalizations (Gerhardt, 1994). If reproductive displacement occurs, differences in female preferences and male characteristics in a

contact zone could result in a divergence of the male calls and the preference of the females (Márquez and Bosch, 1997).

DFA and cluster analysis considering all studied populations showed an evident divergence between the syntopic population of La Escondida and the other diploid populations, even with those that were geographically close to it (fig. 3 and 4). This result is reinforced by the DFA including three population groups (far and near allopatrics and syntopic population), which showed a higher acoustic differentiation among syntopic population and allopatric populations of *O. cordobae* (fig. 5B). Thus, these results appear to be consistent with the fact that the acoustic segregation observed between syntopic and allopatric populations could be explained by a displacement of basic components of advertisement call from the individuals living in contact zones regarding their conspecifics living in allopatry. However, the absence of more syntopic zones in our study partially limits this conclusion. There are few well-documented examples in which it is possible to verify the occurrence of character displacement and reinforcement, although most of these examples include displacement on advertisement call characters of different anurans species (e.g. *Hyla ewingi* complex – Littlejohn, 1965; *Pseudacris nigrata* complex – Fouquette, 1975; *Gastrophryne carolinensis*/*G. olivácea* – Loftus-Hills and Littlejohn, 1992; *H. chrysoyelis*/*H. versicolor* – Gerhardt, 1994). Waage (1979) suggested that the possible rarity of displacement of characters could be attributed to the fact that most studies have focused on male courtship signals rather than on female selectivity. According to the classification proposed by Emlen and Oring (1977), the species under study would have a polygynous mating system of male dominance type lek. Given that in this mating system the number of available males per female is quite high, females take the initiative in selecting a partner moving towards the vocalizing male as in most anurans (Salthe and Mecham, 1974). The females can discrim-

inate between different calls and choose males on this basis, thus generating sexual selection on certain call attributes (Ryan and Keddy-Hector, 1992). The results could be explained by a possible change in the female preference in the presence of males of closely related species in order to avoid mismatches.

Gerhardt (1991) suggests that in many species, the typically narrow range of pulse rate within a population is maintained by female choice. This was corroborated by Castellano and Giacoma (1998) through playback experiences with females of the *Bufo viridis* complex. These authors used different acoustic stimuli, varying different parameters of the co- and heterospecific calls, which were offered to females of both species. If a similar phenomenon was happening in the syntopic population studied, females of closely related species would be selecting conspecific males according to the pulse rate of their calls. This property showed differences between species (Martino and Sinsch, 2002) and variation among individuals within the same species. Thus, conspecific recognition and mating preference is a result of the interaction between changes in male and female preferences. This interaction may cause non-random or selective mating, both between and within species (Ryan, 1990). Consequently, receiver preferences (inter and intra-sexual) should be examined in the future to thus investigate hypotheses about the mechanism and direction of acoustic divergence across populations.

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