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Quantitative acoustic analysis of the vocal repertoire of the golden rocket frog (*Anomaloglossus beebei*)

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This study describes the vocal repertoire of the Guyanan golden rocket frog, *Anomaloglossus beebei*, a bromeliad specialist with biparental care. Using multivariate analyses of nine call properties, as well as the occurrence of nonlinear phenomena, three signal types were distinguished—advertisement, courtship, and aggressive calls. Although all three call types were composed of a short series of rapidly repeated pulses, advertisement calls were produced at higher amplitudes and had longer pulse durations than both courtship calls and aggressive calls. Courtship calls exhibited lower dominant frequencies than both advertisement and aggressive calls, which had similar dominant frequencies. Aggressive calls had more pulses per call, had longer intervals between calls, and occasionally contained one or two introductory pulses preceding the pulsed call. Several acoustic properties predicted aspects of the signaler's body size and condition. Our study demonstrates the reliability of human observers to differentiate the multiple call types of *A. beebei* based on hearing calls and observing the social context in which they are produced under field conditions.
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I. INTRODUCTION

Our objective in this study was to provide statistical descriptions of the vocalizations of the Guyanan golden rocket frog, *Anomaloglossus beebei* (Dendrobatidae). Similar to most other frogs (reviewed in Gerhardt and Huber, 2002; Wells, 2007), male vocalizations play important roles in the reproductive behavior of the golden rocket frog (Bourne *et al*., 2001). In contrast to most other frogs, however, this species is a territorial bromeliad specialist and both sexes provide parental care for their eggs and developing tadpoles (Bourne *et al*., 2001). Consequently, acoustic displays by males of this species may contain information not only on things like species identity, size, fighting ability, and condition, as in other frogs (Gerhardt and Huber, 2002; Wells, 2007), but also on a male's parental care quality, as in some birds (Welling *et al*., 1997; Buchanan and Catchpole, 2000). This frog species, therefore, offers a valuable opportunity to investigate aspects of communication not normally considered in studies of anuran amphibians.

A previous study of vocal communication and reproduction in golden rocket frogs *qualitatively* identified three call types by ear and visual inspection of spectrograms (Bourne *et al*., 2001). All three call types are composed of a short series of rapidly repeated pulses. During courtship and pair formation, males attract females to their territories with advertisement calls. Once a female approaches males switch to courtship calls, which they produce periodically throughout the entire courtship. Males also use an aggressive call in defense of territories against intrusion by conspecific males. Continued investigation into the role of communication in the behavior of golden rocket frogs requires more rigorous, *quantitative* descriptions of their vocalizations and vocal behavior. Our aims in this study were to: (i) Characterize the vocal repertoire of male golden rocket frogs with no *a priori* information using multivariate statistical analyses of call properties, (ii) provide detailed descriptions of each delimited call type, (iii) evaluate relationships between the body size and condition of signalers and acoustic properties of their calls, and (iv) describe temporal patterns of call production.

II. METHODS

A. Study organism and study site

The golden rocket frog is endemic to Guyana, South America, where it is found only on the Kaieteur plateau (∼450 m elevation) in Kaieteur National Park (05°10′N, 59°29′W) (Kok *et al*., 2006). Our study site consisted of an ∼100 ha area adjacent to the upper rim of the plateau near Kaieteur Falls, which creates a mist that appears to influence golden rocket frog densities (Bourne *et al*., 2001). This species lives and breeds almost exclusively on the giant terrestrial bromeliad *Brocchinia micrantha*, where both males and females maintain territories that contain multiple oviposition and tadpole rearing sites comprised of small pools of water that collect in the leaf axils.

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B. Acoustic recordings and analyses

Between May and July, 2007, we recorded a total of 916 calls from 60 males (between 6 and 19 calls/male). Frogs were recorded in the morning from 0600 to 0900 hours (h) and in the late afternoon from 1600 to 1800 h. During a recording session, we subjectively classified the focal male as producing one of three call types by ear and by visual assessment of the social context in which the calls were produced based on previous descriptions by Bourne *et al*. (2001). We classified males as producing advertisement calls ($N = 40$) when they were observed calling antiphonally with other nearby males or when they were calling singly. We classified males as producing courtship calls ($N = 12$) when they were occupying the same leaf axil as an approaching female and aggressive calls ($N = 8$) when their calling behavior was clearly directed toward a conspecific male within 0.5 m that appeared to be intruding into their territory. These initial, subjective classifications provided a basis for comparing the accuracy of observer call type assignments made in the field in real time to those based on detailed acoustical analyses and blind statistical methods.

Sound recordings were made using a Marantz PMD670 solid-state recorder (44.1 kHz sample rate, 16-bit resolution; Marantz America, Inc., Mahwah, NJ) and a handheld Sennheiser ME66 directional microphone (sensitivity: 50 mV/Pa, frequency range: 40 Hz–20 kHz $\pm$ 2.5 dB; Sennheiser Electronic Corporation, Old Lyme, CT). The tip of the microphone was held 1 m $\pm$ 10 cm from calling males during a recording session. Measurements of absolute maximum sound pressure level (dB SPL re 20 μPa, fast RMS, C-weighted, 0.1 dB resolution) were made simultaneously with the acoustic recordings using an Extech 407764 or an Extech 407730 digital sound level meter (Extech Instruments, Waltham, MD). Sound pressure measurements were taken for three calls during a recording session.

At the end of a recording session, we captured the male when possible, measured its mass with a portable spring scale (to the nearest 0.05 g) and snout-to-vent length (SVL) with dial calipers (to the nearest 0.1 mm), and we clipped a unique number of its toes for individual identification. We measured and marked 35 of the 60 recorded males and found an average individual mass of 0.44 g (range: 0.25–0.65 g) and an average SVL of 17.0 mm (range: 15.0–18.5 mm). Following Baker (1992), we computed an index of body condition (i.e., size-independent body mass) as the residuals from a linear regression of the cube root of mass on SVL divided by SVL. To remove the risk of re-recording an unmarked male, we never recorded more than one male within a 200 m^2 area, an area greater than any known male territory (B. A. Pettitt, unpublished). We also measured air temperature with a resolution of 0.1 °C at the position from which the male had been calling using an Oakton digital thermometer (Oakton Instruments, Vernon Hills, ILA); temperatures ranged between 22.0 °C and 29.6 °C with a mean of 24.6 °C.

We used the automatic recognition tool of Avisoft-SASLab Pro v1.5 (Avisoft Bioacoustics, Berlin, Germany) to quantify the following eight spectral and temporal properties for the first pulse and its subsequent interval for each recorded call (Fig. 1)—dominant frequency (kHz), pulse duration (ms; onset to offset), pulse interval (ms; offset to onset), pulse rate (pulses/s; determined as 1/pulse period, where pulse period was the time between the onsets of two consecutive pulses in a call), call duration (ms; onset of first pulse to offset of last pulse), call interval (sec; offset to onset), call rate (calls/min), and pulses per call. We generated call spectrograms by applying a 512-point fast Fourier transform (FFT) with a Hanning window. We selected the first pulse for these analyses for two reasons. First, visual inspections of recordings suggested variation in the interval

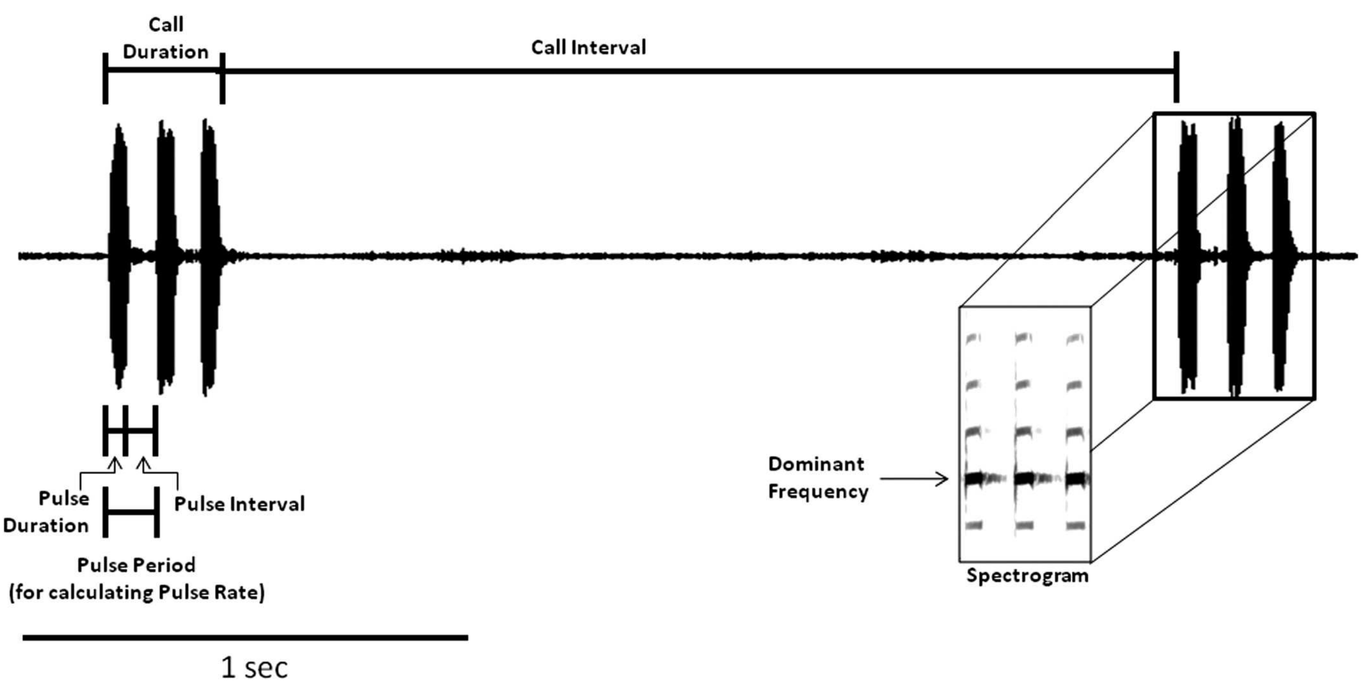

FIG. 1. Waveform and spectrogram (see inset) of an *Anomaloglossus bebeei* advertisement call showing the spectral call property and five of the temporal call properties quantified in this study. Pulse rate was calculated as 1/pulse period and call rate was calculated as the number of calls delivered per minute.

following the initial pulse differed among some call types; specifically, aggressive calls appeared to consist occasionally of an introductory pulse followed by a longer pulse interval and a subsequent series of rapidly repeated pulses. Second, preliminary analyses suggested that the effect sizes for differences in pulse characteristics within a call were small ($0.03 \leq \eta^2 \leq 0.07$ for pulse duration, pulse interval, pulse rate, and pulse frequency), indicating that analyses of first pulses captured the relevant variation among call types.

We also examined separately the occurrence of nonlinear phenomena by visually inspecting spectrograms of all verified calls. Three types of nonlinear events were quantified—subharmonics, deterministic chaos, and frequency jumps (Riede *et al.*, 2004). Subharmonics are additional spectral components that are integer fractions of the fundamental frequency, f_0 (e.g., $f_0/2$, $f_0/3$), and can suddenly appear as "subbands" evenly spaced between adjacent harmonics. Deterministic chaos is considered a broadband segment of nonrandom noise with no identifiable harmonics in the spectrum. Frequency jumps are sudden and abrupt changes in the fundamental frequency and corresponding harmonics (Fig. 2). With each type of nonlinear event, we calculated the frequencies with which they occurred at the beginning, middle, and end of each call. We defined the beginning as the first pulse, the middle as any pulse between the first and last pulses and the end as the last pulse of a call.

C. Statistical analyses of vocalizations

All statistical analyses were conducted using SPSS v12.0 (SPSS, Inc., Chicago, IL) and we used a significance criterion of $\alpha = 0.05$ for all tests.

1. Data transformations and temperature corrections

We created a data set describing nine variables for each individual male corresponding to the mean value of each of the eight analyzed call properties (determined over all calls recorded from the male) and the mean sound pressure level (in dB SPL) of his calls (determined over three calls). We then subdivided this data set into three "call type data sets"

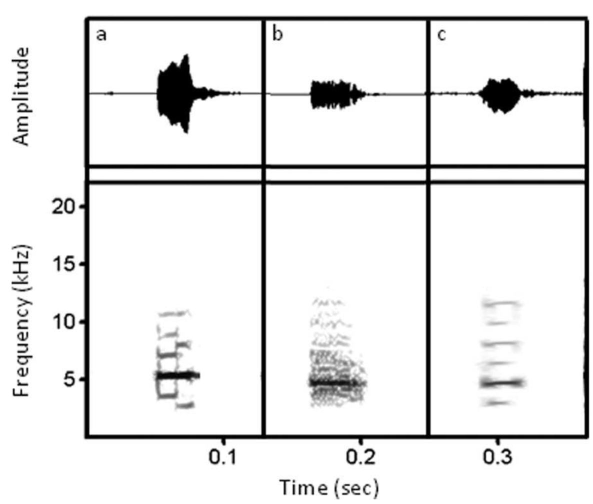

FIG. 2. Spectrogram and waveform of the three types of nonlinear phenomena found in *Anomaloglossus beebei* vocalizations: (a) Frequency jumps, (b) deterministic chaos, and (c) subharmonics.

according to the type of call subjectively attributed to the individual at the time of recording based on social context (following Bourne *et al.*, 2001). We tested the normality of each of the nine call properties in each call type data set using Shapiro-Wilk tests. Of the 27 possible combinations of 9 call properties $\times$ 3 call types, 18 were normal and 9 were not. We found normalizing transformations for six of nine non-normal properties; however, we were unable to normalize mean call frequency for males classified as producing advertisement calls and mean number of pulses per call for males classified as producing both advertisement calls and courtship calls. We analyzed sound pressure on a logarithmic (dB) scale because a conversion to a linear scale (μPa) resulted in a non-normal data set in which normality could be achieved only through a log transformation.

We adjusted call properties correlated with temperature (Appendix A) to a standard value of 24 °C, which was close to the mean air temperature of all recordings (24.6 °C), using least squares linear regression following Platz and Forester (1988). We adjusted a call property if the correlation between that property and temperature was greater than 0.355, the minimum significant r-value for our largest call type data set ($N = 40$). There was no difference in air temperature values among the three call type data sets [one-way analysis of variance (ANOVA), $F_{2,59} = 0.68$, $P = 0.51$].

Transformed, temperature-corrected values for individual means were used in all subsequent statistical analyses. The results reported in the following were qualitatively unchanged in separate analyses conducted using calls that were transformed to improve normality and corrected for temperature before subdividing the entire data set into three call type data sets. Hence, data transformations and temperature corrections are not responsible for differences among call types reported here.

2. Multivariate analyses

We used a combination of multivariate statistical approaches to describe the golden rocket frog vocal repertoire and to assess the accuracy of our subjective call type classifications made at the time of recording. For these analyses, we standardized individual mean values of call properties ($N = 60$ males) by generating standardized Z scores to equally weight properties measured in different units (e.g., kHz and s). These standardized values, many of which were highly correlated (see Appendix B), were used as input variables in a principal components analysis (PCA) to reduce the total number of predictor variables. We extracted all principal components with eigenvalues greater than 1.0 and used the corresponding principal component scores as input variables in a cluster analysis (CA) and discriminant function analysis (DFA).

We performed the CA to assess natural groupings in the males we recorded by blindly analyzing the shared attributes of their calls' acoustic properties and to determine an appropriate number of call type categories. We performed a hierarchical CA where similarities between males were estimated using the squared Euclidean distance measure and clusters were combined using the average linkage between-groups method (Terhune *et al.*, 1993). The hierarchical clustering solution was represented by a dendrogram.

We used a DFA to verify our initial classification of males and to determine which call properties contributed most to the differentiation of call types. The accuracy of the DFA model was tested using cross validation, a process that classifies each case while leaving it out from the model calculations. The accuracy of our initial classification was tested by evaluating the ability of the DFA to classify a male correctly into the same call type data set (advertisement, courtship, or aggressive) to which we had subjectively assigned it based on social context. Due to unequal sample sizes among call type data sets, a chance-corrected procedure (Cohen's κ statistic) was used to determine if classification success was better than chance (Titus *et al.*, 1984). Individual males for which our subjective call type classifications were not verified by multivariate analyses were removed from subsequent statistical analyses.

3. Descriptions and comparisons of call types

We report overall median values of all call properties and compared differences in these medians among the three groups of males producing different call types with Kruskal Wallis tests. When these tests yielded significant results, we performed *post hoc* pairwise comparisons of all three pairs following Siegel and Castellan (1988). We examined the difference in the occurrence of nonlinear phenomena among the call types using χ-squared tests.

4. Relationships with size and condition

For each call type data set, we calculated Pearson's correlation coefficients to describe the relationship between the individual means of each call property and body size (mass and SVL) and condition. We calculated correlation coefficients for each call type data set separately because when we pooled all 60 males into a combined data set, six of the nine call properties had bimodal or highly skewed, non-normal distributions. Associations were considered significant when the correlation between a call property and body size or condition was greater than 0.407, the minimum significant r-value for our largest call type data set ($N = 24$). Because these analyses were exploratory (and not confirmatory) in nature, we did not correct for multiple statistical comparisons.

D. Temporal analyses of call type use

We monitored male calling activity from 0600 to 1800 h for 24 sampling days between July and August 2008. For each sampling day, we visited eight locations along a 0.71 km transect that followed a trail along the edge of the Kaieteur plateau. The trail passed through an area with the highest concentration of golden rocket frogs and, likewise, each of the eight locations was selected for its high local density of calling males. The number of males calling at a site typically varied between one and five. We stopped at all eight locations within a 30 min period and counted the number of males heard producing advertisement and aggressive calls within a 1 min sampling period at each location. Our accuracy in differentiating between these two call types in the field was confirmed by results described in the following. If a male switched call types during the 1 min sampling period, we counted that male in terms of the first call type produced. On each sampling day, we conducted this census four times at each of the eight selected locations, one time during each 3 h time window between 0600 and 1800 h (e.g., once during 0600–0900, once during 0900–1200, etc.). Two observers conducted censuses; each ran the transect four times per day every other day. To ensure the reliability of observations, we conducted concurrent counts (both observers counting calling males separately) at all eight locations over 3 d with minimal discrepancy (i.e., occasional differences of 1, rarely 2 males).

III. RESULTS

A. Multivariate analyses

1. Principal components analysis

The first two components had eigenvalues greater than 1.0 and accounted for 76.9% of the original variation (Table I). In a two-dimensional space representing the first two principal components, the males we subjectively classified as producing advertisement calls, courtship calls, or aggressive calls at the time of recording generally grouped into three separate clusters (Fig. 3). Principal component 1, which explained 54.6% of the variance, was most closely correlated with several temporal call properties that included call duration, the number of pulses per call, call interval, call rate, and pulse interval (Table I). This component distinguished males that we initially categorized as producing aggressive calls from those that were classified as producing courtship calls and advertisement calls. Principal component 2 explained an additional 22.3% of the variance and was strongly correlated with dominant frequency, call amplitude, and pulse duration (Table I). Component 2 readily distinguished most of the males that we initially categorized as producing courtship calls from those that we categorized as producing aggressive calls and advertisement calls (Fig. 3).

Within this two-dimensional space, three individuals (males: 5, 15, and 18) fell outside of the call type grouping to which we initially assigned them. Mean call duration,

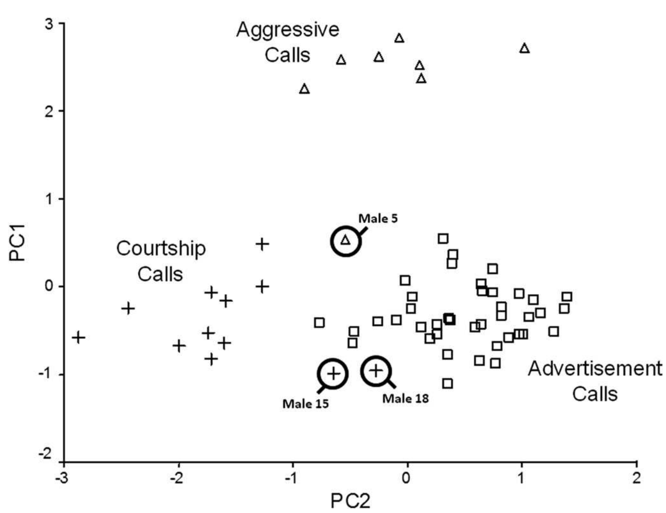

FIG. 3. Mean scores for the first two principal components for each proposed signal type as determined by subjective analyses ($N = 60$ males). We initially classified males as producing advertisement (□), courtship (+), or aggressive (△) calls.

TABLE I. Factor loadings of the first two principal components using standardized, temperature-corrected input variables.[a]

Factor	PC1	PC2
Pulse duration	−0.562	**0.645**
Pulse interval	**−0.825**	−0.169
Pulse rate	−0.513	−0.591
Call duration	**0.944**	−0.040
Call interval	**0.889**	0.008
Call rate	**0.936**	0.094
Pulses per call	**0.940**	−0.139
Dominant freq	0.321	**0.769**
dB	−0.330	**0.772**
Variance (%)	54.57	22.34
Cumulative variance (%)	54.57	76.91

[a]Loading values exceeding 0.6 are highlighted in boldface.

number of pulses per call, and pulse interval were lower than average for male 5 and as a result, this male fell between the grouping of aggressive calls and advertisement calls along the component 1 axis. Males 15 and 18, both initially classified as producing courtship calls, had much higher call amplitudes (79.3 and 79.0 dB SPL, respectively) compared to that averaged over the 10 remaining males classified as producing courtship calls (69.3 dB SPL). Consequently, these two males fell between the grouping of courtship calls and advertisement calls along the component 2 axis (Fig. 3).

2. Cluster analysis

The CA revealed three main clusters that corresponded almost identically to our initial classifications (Fig. 4). All individuals grouped into one of these three clusters. There were three grouping errors involving males that we originally classified as giving aggressive calls (male 5) or courtship calls (males 15, 18), but were grouped by the CA with other males that we classified as producing advertisement calls.

3. Discriminant function analysis

The DFA correctly classified 93.3% of males as belonging to the call-type category to which they had been assigned based on our initial subjective judgments. This level of correct classification was significantly greater than the mean *a priori* probability of 33.3% correct classification expected by chance (paired t-test, $t_2 = 20.32$, $P = 0.002$; Cohen's κ, $\kappa = 0.89$). Across call types, classification success ranged from 83.3% for courtship calls (10 of 12 males correctly classified) to 87.5% for aggressive calls (7 of 8 males correctly classified) and 97.5% for advertisement calls (39 of 40 males correctly classified). Similar to results from the PCA and CA, male 5 and males 15 and 18 were statistically classified by the DFA as producing advertisement calls, whereas we had subjectively classified them as producing aggressive and courtship calls, respectively. Unlike previous results, male 28 was classified as producing courtship calls in the DFA, but was classified as producing advertisement calls both subjectively and in the CA. This individual's calls had somewhat lower than average amplitude and pulse duration (79.8 dB and 30.1 ms; two properties that loaded heavily on PC2), but these values still fell within the range for all other males producing advertisement calls (Table II). The following descriptions and comparisons of call types are based on the calls of males whose call type was verified statistically ($N = 57$); males 5, 15, and 18 were removed, while male 28 was included because he was classified by the CA and our original aural and visual categorizations as producing advertisement calls.

B. Descriptions and comparisons of call types

All three call types (Fig. 5; Table II) are composed of a series of short pulses (25–40 ms each) produced at rates of ∼10–12 pulses s^{-1}. Each pulse comprises multiple harmonics, with the second harmonic (typically 4–6 kHz) being the dominant frequency. There were significant differences

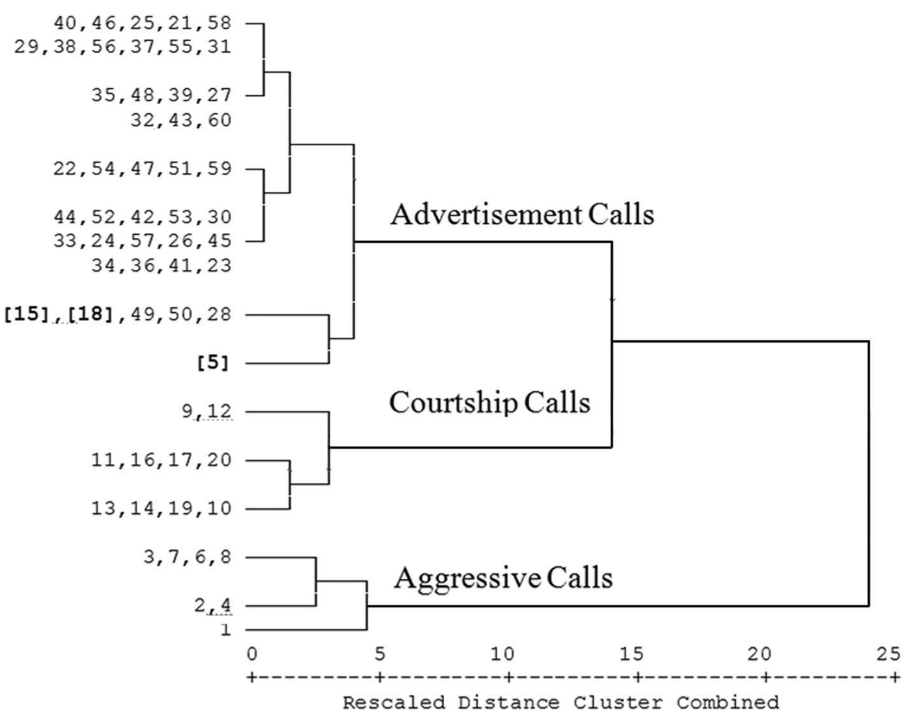

FIG. 4. A cluster analysis using mean values for call properties from recordings of 60 males. Numbers listed on the left-hand side of the dendrogram correspond to male ID codes. All individuals grouped into one of three clusters: advertisement, courtship, or aggressive calls. Based on our initial visual and aural classifications, there were three grouping errors (in bold and brackets) involving males that we originally classified as giving aggressive calls (male 5) or courtship calls (males 15, 18).

TABLE II. Physical characteristics of the vocalizations of adult males of *Anomaloglossus beebei*.[a,b]

Vocalization type (N)	Dominant frequency (kHz)***	dB (SPL) at 1 m***	Call properties: Pulses per call***	Call duration (ms)***	Call interval (s)***	Call rate (calls/min)***	Pulse properties: Pulse duration (ms)***	Pulse interval (ms)***	Pulse rate (pulses/s)**
Advertisement	5.42^A	83.1^A	3.10^A	231^A	2.15^A	26.1^A	35.4^A	53.3^A	11.3^A
(40 males;	[5.30–5.55]	[80.3–86.3]	[3.00–3.74]	[208–280]	[1.93–2.40]	[23.7–28.6]	[33.8–37.4]	[51.3–57.0]	[10.7–11.8]
19 calls/male)	(4.63–5.73)	(74.8–90.2)	(2.50–4.30)	(180–352)	(1.33–2.87)	(19.7–41.5)	(29.4–41.8)	(46.6–63.6)	(10.1–12.6)
Courtship	3.97^B	70.8^B	3.40^A	225^A	1.91^A	31.9^A	31.0^B	53.0^A	11.9^B
(10 males; 9 calls/male)	[3.92–4.23]	[66.7–72.6]	[3.18–4.10]	[200–295]	[1.75–2.39]	[26.0–35.3]	[28.6–32.2]	[50.8–55.2]	[11.7–12.2]
	(3.75–4.59)	(59.6–72.8)	(3.10–5.30)	(188–377)	(1.60–3.40)	(20.4–38.5)	(25.3–33.8)	(45.6–63.3)	(10.9–12.8)
Aggressive	5.55^A	75.8^B	8.06^B	798^B	5.94^B	12.6^B	27.9^B	82.9^B	10.6^C
(7 males; 6 calls/male)	[5.52–5.72]	[70.7–78.0]	[7.68–8.46]	[693–877]	[3.91–7.13]	[11.4–13.9]	[25.2–32.0]	[70.3–93.1]	[10.5–11.0]
	(5.16–5.97)	(63.7–79.9)	(7.26–8.94)	(650–941)	(2.92–9.29)	(9.7–16.2)	(24.9–32.0)	(66.5–96.0)	(9.8–11.1)

[a]Values are expressed as median, interquartile range (in square brackets), and range (in parentheses). Median values represent the medians of individual means, which were determined by averaging over all calls recorded from an individual. Medians with different superscripts (A, B, C) are significantly different ($P < 0.05$) based on *post hoc* tests following Siegel and Castellan (1988). The three males for which we apparently made initial classification errors (5, 15, and 18) were removed from these descriptive analyses.

[b]Kruskal Wallis test, ** $P < 0.01$, *** $P < 0.001$.

among the three call types in all nine of the acoustic properties measured ($15.0 < H < 32.2$; $0.0001 < P < 0.001$; all $df = 2$; Table II). Advertisement calls were produced at higher amplitudes and had longer pulse durations (medians = 83.1 dB SPL and 35.4 ms) than both courtship calls (70.8 dB SPL and 31.0 ms) and aggressive calls (75.8 dB SPL and 27.9 ms), which did not differ in these two properties. Courtship calls had lower dominant frequencies (median = 3.97 kHz) than both advertisement calls and aggressive calls, which had similar dominant frequencies (5.42 and 5.55 kHz, respectively). Compared with other call types, aggressive calls had more pulses per call (median = 8.06) and had longer intervals between calls (5.94 s). Advertisement and courtship calls did not differ in these respects. Additionally, aggressive calls occasionally (13 of 42 calls) contained one or two introductory pulses followed by a longer pulse interval (range: 228–1842 ms) and an additional 6–9 pulses, a temporal pattern not seen in other call types (Fig. 5).

Courtship calls had a higher occurrence of nonlinear phenomena (89%; 330/370 pulses) than did either advertisement calls (<1%; 11/2679 pulses) or aggressive calls (5%; 18/392 pulses; χ^2 (2, $N = 3241$) = 1431.8, $P < 0.001$). As illustrated in Fig. 2, these phenomena included: frequency jumps (10.0% of calls containing nonlinear phenomena), deterministic chaos (29.9%) and subharmonics (60.1%). Both subharmonics and deterministic chaos, but not frequency jumps, occurred in advertisement calls. Only deterministic chaos occurred in aggressive calls. Courtship calls included all three nonlinear phenomena. Frequency jumps were equally likely to occur in the pulses at the beginning, middle, and end of calls [χ^2 (2, $N = 38$) = 3.21, $P = 0.20$]. The onsets of abrupt frequency changes typically occurred about halfway into the pulse (mean start time within a pulse = 0.017 ms; mean pulse duration = 0.033 ms) and exhibited an average change in frequency of −632 Hz. Pulses exhibiting deterministic chaos were more often found in the middle or at the end of calls (92/114; 92%) than at the beginning [χ^2 (2, $N = 114$) = 29.11, $P < 0.001$]. Within pulses, chaos occurred at the initiation of a pulse (14/114; 12%), the end of a pulse (39/114; 34%) or throughout the entire pulse (61/114; 54%) with an average duration of 0.029 ± 0.010 ms (standard deviation; mean pulse duration = 0.038 ms). Subharmonics were only observed to occur in the $f_0/2$ pattern. They were found at the beginning, middle, and end of calls, but were most often found in the middle [113/229, 49%; χ^2 (2, $N = 229$) = 26.52, $P < 0.001$]. Although subharmonics are often found to be precursors to deterministic chaos (e.g., Wilden *et al.*, 1998; Riede *et al.*, 2004), our study found these nonlinear events to occur most often on their own. Subharmonics as precursors to chaos occurred in only 13 of the 229 pulses with subharmonics (5.7%).

C. Relationships with size and condition

We found significant correlations between call properties and SVL, mass, or condition for all three call types (Table III). In general, advertisement calls and courtship calls were not that informative about SVL and mass. Larger males produce both call types with lower pulse rates and courtship

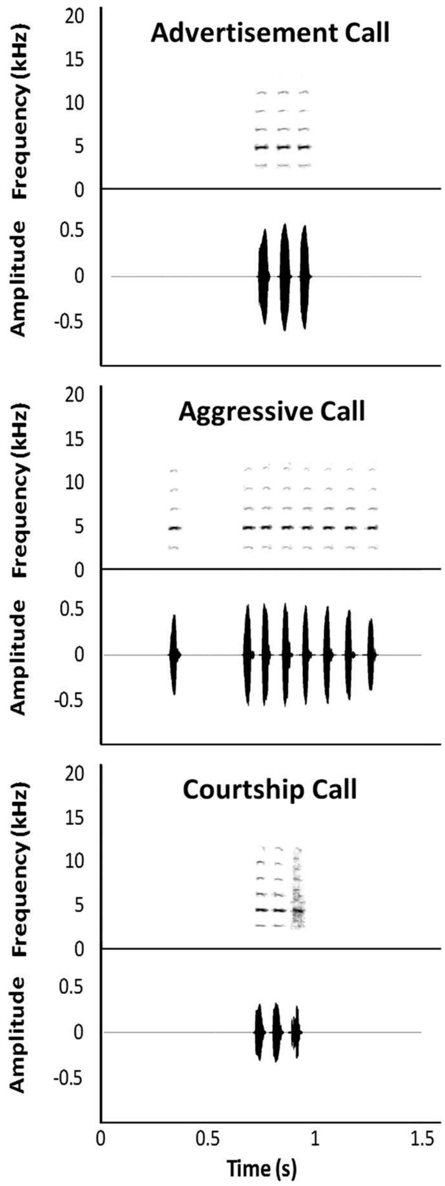

FIG. 5. Spectrogram (FFT = 512 points, Hanning window, 75% overlap) and waveform of a typical (i) advertisement call, (ii) aggressive call, and (iii) courtship call of male *Anomaloglossus beebei* from Kaieteur National Park, Guyana. Air temperature during recordings was 26.1, 25.2, and 23.4 °C, respectively.

calls with longer pulse durations. Two properties of advertisement calls, but none of courtship calls, were significantly correlated with our measure of size-independent condition. Males in better condition produced advertisement calls at faster rates and higher sound pressure levels (Table III). Compared with advertisement calls and courtship calls, aggressive calls appeared more informative about caller size and condition; all aggressive call properties, except the number of pulses per call, were correlated with SVL, mass, or condition, or all three (Table III). Interestingly, aggressive calls were the only call type for which dominant frequency was correlated (negatively) with body size.

D. Temporal patterns of call type use

Males rarely called prior to sunrise [~0545–0600 h [B. A. Pettitt (unpublished)], but almost half (45%) of all calls were produced between 0600 and 0900 h (Fig. 6). Males continued to call throughout the day with calling behavior waning after sunset [~1800–1815 h; B. A. Pettitt (unpublished)]. Between 0600 and 0900 h, 92% of calling males produced advertisement calls, and this percentage slowly decreased throughout the day to 14% between 1500 and 1800 h (Fig. 6). On the other hand, calling males produced aggressive calls primarily after 0900 h with the bulk of aggressive calls being produced between 1200 and 1800 h (Fig. 6).

IV. DISCUSSION

Our quantitative categorization of golden rocket frog vocalizations based on multivariate statistical analyses confirmed our initial subjective classifications of vocalizations into three distinct call types: advertisement, courtship, and aggressive calls. Although all three call types shared some acoustic similarities, they also exhibited reliable differences in several acoustic properties, including amplitude, dominant frequency, call duration, call rate, and the occurrence of nonlinear phenomena. Advertisement calls were the most commonly heard vocalization and, on average, these calls were produced at higher sound pressure levels than either courtship or aggressive calls. Courtship calls closely resemble advertisement calls; however they were typically produced at lower frequencies and amplitudes and contained more nonlinear phenomena (Fig. 5). These quantitative findings are consistent with the qualitative description detailed in Bourne *et al.* (2001) describing the decreases in amplitude and frequency heard in a male's vocalizations when a female approaches. Aggressive calls also resembled advertisement calls, but had longer call durations and faster call rates and were occasionally initiated by an introductory pulse (Fig. 5).

Vocal repertoires consisting of three call types produced in similar social contexts have been noted in other closely related dendrobatids [e.g., *Anomaloglossus stepheni* (Juncá, 1998); *Rheobates palmatus* (Lüddecke, 1999); *Allobates caeruleodactylus* (Lima *et al.*, 2002)]. Similar to *A. beebei*, *R. palmatus* produce courtship and aggressive calls at lower sound amplitudes; however, advertisement calls appear to have longer call durations (Lüddecke, 1999). *A. caeruleodactylus* also produces a soft courtship call, but the calls used when a territorial intruder approaches are loud, yet distinct from advertisement calls (Lima *et al.*, 2002). *Allobates marchesianus* exhibits three call types; however, the repertoire of this species consists of one low intensity courtship call and two high intensity advertisement calls that differ temporally (i.e., discrete or continuous) and in how they are used in different social contexts. Specifically, one call type was produced only during territorial advertisement, whereas the other was used during territorial advertisement, agonistic interactions, and courtship (Lima and Keller, 2003). *Mannophryne trinitatis*, on the other hand, produces one type of advertisement call and at least two types of courtship calls, but lacked an aggressive call (Wells, 1980).

Although descriptions of the occurrence of nonlinear phenomena in the vocal repertoires of anurans are rare, such features have now been documented in vocalizations of *A. beebei* (this study) as well as the concave-eared torrent frog (*Odorrana tormota*), an anuran known for its unusually large

TABLE III. Pearson correlation coefficients between call properties and mass, SVL and body condition in male *A. beebei*.[a,b]

	Advertisement calls ($N=24$)			Courtship calls ($N=7$)			Aggressive calls ($N=4$)		
	SVL	Mass	Cond.	SVL	Mass	Cond.	SVL	Mass	Cond.
Pulse duration (ms)	0.117	0.214	0.138	0.006	**0.622**	0.286	0.065	−0.176	**−0.714**
Pulse interval (ms)	0.346	−0.014	−0.367	0.100	0.000	−0.044	**−0.722**	**−0.795**	0.133
Pulse rate (pulses/s)	**−0.407***	−0.150	0.229	**−0.460**	−0.236	0.186	0.298	0.074	**−0.751**
Call duration (ms)	0.293	0.058	−0.218	0.191	0.055	−0.069	**−0.908**	**−0.983***	0.125
Call interval (s)	0.066	−0.264	−0.401	0.077	0.109	0.029	**0.790**	**0.739**	**−0.409**
Call rate (calls/min)	−0.157	0.266	**0.508***	−0.047	−0.078	−0.023	−0.361	**−0.417**	−0.126
Pulses per call	0.190	−0.064	−0.257	0.226	−0.117	−0.180	−0.327	−0.253	−0.258
Dominant freq (kHz)	−0.181	−0.146	0.031	0.269	0.136	−0.086	**−0.815**	**−0.629**	**0.889**
SPL (dB)	−0.328	0.154	**0.526****	0.058	0.234	0.047	**0.443**	0.395	−0.230

[a]Pearson's correlation coefficients, * $P < 0.05$, ** $P < 0.01$.
[b]Boldface values indicate significant associations (see text for explanation).

call repertoire (Suthers *et al.*, 2006). In *O. tormota*, various combinations of nonlinear phenomena are common and may facilitate individual discrimination (Feng *et al.*, 2009). Future playback experiments are required to determine if a similar function may also occur in *A. beebei*, in which males also discriminate between the calls of strangers and territorial neighbors (Bourne *et al.*, 2001).

In the present study, we found no correlation between either size or condition and the call duration or dominant frequency of advertisement calls, suggesting that neither trait functions as a size or condition-assessment signal in *A. beebei*.

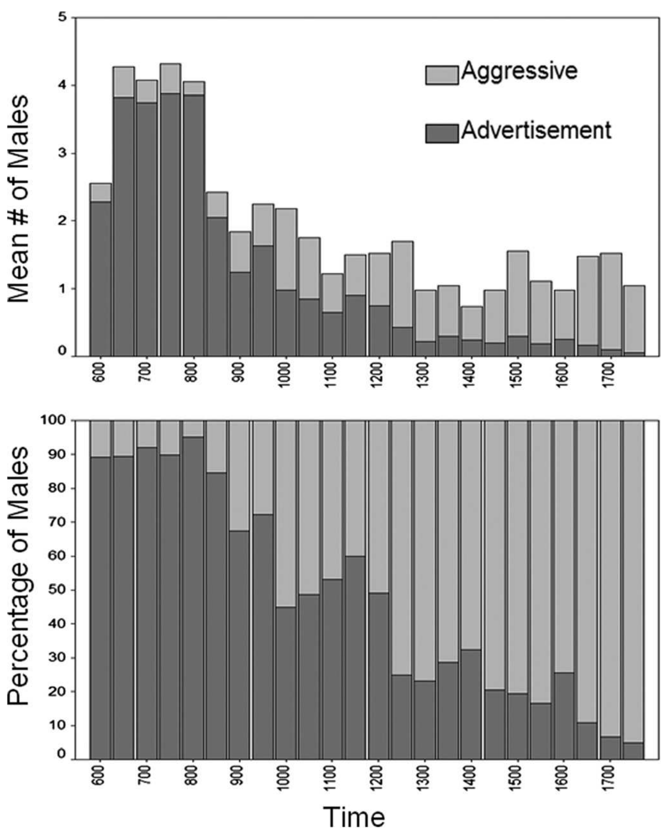

FIG. 6. The diel pattern of advertisement (dark gray) and aggressive (light gray) calls produced by *Anomaloglossus beebei* during 24 sampling days in July–August 2008, in Kaieteur National Park, Guyana. Each bar represents the mean number (A) or the relative proportions (B) of calling males during each 30 min period from 0600 to 1800 h.

We did find both the rate and amplitude of advertisement calls to be positively correlated with condition. These results were not surprising given that both traits are considered to be condition-dependent and suggest the hypothesis that the rate and amplitude of advertisement calls reflect male quality in *A. beebei*. Because male golden rocket frogs provide parental care, including guarding eggs and transporting tadpoles (Bourne *et al.*, 2001), a next step will be to assess whether males in better condition provide better care for their offspring. If so, then it could be revealing to test the hypothesis that females assess male condition (and hence his parental care abilities) based on properties of his advertisement or courtship calls.

In terms of aggressive calls, our results indicate that this call type has the potential to effectively communicate a caller's size, fighting ability, and physiological condition. As almost all properties of aggressive calls were correlated with body size, condition, or both, these calls may provide receivers with multiple, potentially redundant signals by which to assess the caller. For instance, larger males may be identified by aggressive calls with lower dominant frequencies, greater amplitudes, shorter calls, and slower call rates. It will be important in future studies to test the hypothesis that size and condition related information in aggressive calls influences behavioral decisions during agonistic encounters (Gerhardt and Bee, 2007).

Calling activity by male golden rocket frogs peaked between 0600 and 0900 h and declined thereafter. Studies of diel variation in vocal behavior in some dendrobatids found similar patterns of maximum call activity occurring during morning hours (Pröhl, 1997; Graves, 1999; Hermans *et al.*, 2002). In contrast, a two-phase calling period, with peaks in the morning and the late afternoon or evening, has been noted in other dendrobatids (Stewart and Rand, 1992; Juncá, 1998). Interestingly, advertisement and aggressive calls had different patterns of usage throughout the day in golden rocket frogs (Fig. 6); the former were produced primarily during morning hours while the latter became more frequent (both relatively and absolutely) later in the day. This temporal variation in call type use differs from that demonstrated in other diurnal anurans. *Eleutherodactylus coqui*, for example, produced both advertisement and aggressive calls in the morning and evening, however, at dawn males produce advertisement calls at first light, whereas aggressive calls were heard ~45 min later when advertisement calling was waning (Stewart and Rand, 1992). This pattern reversed in the evening when males produced predominantly aggressive calls in the early afternoon and then switched to advertisement calls at dusk (Stewart and Rand, 1992). *Hylodes heyeri* males vocalized throughout the day, but produced more aggressive calls at sunrise and sunset and more advertisement calls during the day (Lingnau and Bastos, 2007).

In summary, our results provide quantitative evidence that the golden rocket frog's vocal repertoire is made up of three call types that can be reliably distinguished in the field. We found these call types to differ in all call properties examined as well as temporal patterns of use. Further, our results suggest that calls provide sufficient information for receivers to potentially predict a caller's body size and condition from various advertisement, courtship, and aggressive call properties. Future work should investigate the extent to which such information might be used by males to assess fighting ability or females to assess the quality of a mate. Because of this species' unique biparental care behavior, our findings also provide an important foundation for future investigations into the potential for *A. beebei* vocalizations to convey information on a male's parental care quality. Such studies could provide much needed empirical evidence to help close a current gap in our understanding of mate selection in anurans related to whether there are reliable acoustic indicators of direct fitness benefits to females.

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APPENDIX A

See Table IV.

TABLE IV. Correlations with temperature.[a,b]

	Advertisement calls ($N = 40$)	Courtship calls ($N = 12$)	Aggressive calls ($N = 8$)
Pulse duration (ms)	**−0.374***	**−0.362**	−0.102
Pulse interval (ms)	**−0.372***	−0.349	**0.704**
Pulse rate (pulses/s)	**0.649****	**0.616***	0.045
Call duration (ms)	−0.267	0.150	**0.388**
Call interval (s)	**−0.456****	−0.252	**−0.469**
Call rate (calls/min)	**0.355***	0.197	0.113
Pulses per call	−0.041	0.299	**−0.424**
Dominant freq (kHz)	0.022	0.216	**−0.456**
SPL (dB)	0.081	0.035	**0.490**

[a]Pearson's correlation coefficients, * $P < 0.05$, ** $P < 0.01$.
[b]Boldface values indicate call properties corrected for temperature.

APPENDIX B

See Table V.

TABLE V. Call property correlation matrix.[a,b]

	Pulse interval	Pulse rate	Call duration	Call interval	Call rate	Pulses per call	Dominant frequency	SPL
Pulse duration	0.413**	−0.203	−0.492**	−0.459**	−0.453**	−0.589**	0.187	0.522**
Pulse interval		0.636**	−0.731**	−0.627**	−0.709**	−0.668**	−0.276*	0.131
Pulse rate			−0.454**	−0.359**	−0.431**	−0.357**	−0.418**	−0.081
Call duration				0.771**	0.829**	0.976**	0.268*	−0.350**
Call interval					0.954**	0.787**	0.266*	−0.217
Call rate						0.832**	0.390**	−0.191
Pulses per call							0.239	−0.407**
Dominant freq								0.477**

[a]Pearson's correlation coefficients, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
[b]Matrix calculated using transformed, temperature-corrected, standardized call properties values ($N = 60$ males). Identical results were obtained using transformed, temperature-corrected, unstandardized values.

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