

**Paternal Effort in Relation to Acoustically Mediated Mate Choice in a
Neotropical Frog**

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Dedication

This dissertation is dedicated to my sister Michelle Pettitt. Throughout this education process, Michelle has kept me grounded and appreciative of the important things in life.



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**Introduction: Paternal Effort in Relation to Acoustically Mediated Mate
Selection in a Neotropical Frog**

INTRODUCTION

Studies of anuran acoustic signaling and communication have greatly furthered our understanding of the functions of acoustic signals in sexual selection. Such research has examined variation among signal properties and has identified how this variation is used in both mate selection and male-male interactions. Specifically, acoustic signals may contain information on species identity, size, fighting ability, genetic quality and condition. One aspect of communication not normally considered in studies of anuran amphibians involves the extent to which acoustic signals may indicate the quality of parental care a male provides. My research examines this question in the golden rocket frog (*Anomaloglossus beebei*), a Neotropical dendrobatid that exhibits acoustically mediated mate choice and biparental care.

The golden rocket frog is a little studied species that is found only in Kaieteur National Park in central Guyana, South America. These frogs live and breed almost exclusively in giant tank bromeliads, depositing eggs and tadpoles in the small pools of water or “phytotelmata” that collect within the leaf axils of these plants. Males are territorial and attract females to oviposition sites within their territories using “loud” advertisement calls. Both males and females provide care to the offspring. Males attend egg clutches and transport newly hatched tadpoles to “tadpole-rearing” phytotelmata while females provide trophic eggs to developing tadpoles.

Here I present the results from a series of studies on acoustic variability, mate selection, and parental care behaviors in the golden rocket frog. These studies include

analyses of approximately 1500 acoustic signals, 600 h of behavioral data, and 450 female preference tests. I use the results of these analyses to address questions about call repertoire size, individual variation and distinctiveness in *A. beebei* advertisement calls, the call properties used by female in mate selection, characteristics of female and male parental care including offspring deposition site selection and the relationship between male advertisement call properties and paternal care quality.

In the first chapter, I describe the vocal repertoire of the golden rocket frog using multivariate analyses of nine call properties. I distinguish three call types: advertisement, aggressive and courtship calls. I found several acoustic properties of aggressive calls, in particular, that predicted aspects of the signaler's body size and condition. This study demonstrates the reliability of human observers to differentiate among the three call types based on hearing the calls and observing the social context in which they are produced under field conditions.

In the second chapter, I investigate call variation and examine the relative importance of different advertisement call properties for coding individual indistinctiveness. I quantify patterns of within-individual and among-individual variability in 760 advertisement calls of 40 males as well as repeatability in 16 additional males. Of eight call properties examined, all exhibited significant among-individual variation and moderate to high repeatability. Based on discriminant function analysis, I found that individual male golden rocket frogs can be distinguished statistically by their advertisement calls.

In the third chapter, I examine the abiotic and biotic factors associated with egg and

tadpole deposition site selection and hatching success. I evaluate evidence for my hypothesis that parents that use separate sites for oviposition and tadpole rearing may benefit from using different criteria for selecting suitable habitat for eggs and tadpoles due to their different requirements for growth and survival. I found oviposition sites had higher dissolved oxygen concentrations, more crabs, smaller water volumes, lower temperatures and were found in leaves at lower angles compared to tadpole rearing sites. I also found successful egg clutches were positively associated with dissolved oxygen and the presence of dragonfly larvae as well as nearby conspecifics and negatively associated with water temperature and the presence of predatory crabs.

In the fourth chapter, I test the predictions of four hypotheses proposed to explain the relationship between male advertisement call properties and the quality of care a male provides. The good parent hypothesis predicts that males with more attractive traits will provide more care, while the differential allocation hypothesis predicts that they will provide less care. The trade-off hypothesis also predicts less care, but only when mating opportunities are numerous. The essential male care hypothesis predicts males will reliably signal their parental care quality when male care is essential for offspring survival. To evaluate the predictions associated with each of the four hypotheses I examine: (i) female preference for male advertisement traits, (ii) importance of male care on offspring survival and (iii) the relationships between male attractiveness and male care, female care and opportunities for multiple mates. My results indicate that females prefer longer calls and that male care significantly impacts hatching success and offspring survival. I also found that more attractive males (i.e., males that produce longer calls)

provided higher quality paternal care which is consistent with both the good parent and the essential male care hypotheses. My findings suggest that females may gain direct fitness benefits by selecting males that produce longer advertisement calls.

I would like to note here that each of the following four chapters was written so it could be used independently for publication. I retain the first person plural throughout each chapter because this work is the product of many minds and hands.

**Chapter 1: Quantitative acoustic analysis of the vocal repertoire of the
golden rocket frog (*Anomaloglossus beebei*)**

SUMMARY

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. While 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.

INTRODUCTION

Animals commonly produce multiple types of acoustic signals, often in different behavioral contexts (Kroodsma and Miller, 1996; Bradbury and Vehrencamp, 1998; Gerhardt and Huber, 2002). Statistical analyses of signals and signal use can achieve at least four important goals toward understanding an animal's acoustic communication system. First, multivariate statistical methods can be used to describe a species' vocal repertoire without specific *a priori* hypotheses about the number of different signal types present (e.g., Boisseau, 2005; Knörnschild et al. 2010). Second, subsequent statistical descriptions of each delimited signal type provide valuable information about signal differences and species-specific acoustic properties (e.g., Gamba and Giacoma, 2007). Third, correlation analyses can reveal which acoustic properties would be useful to receivers for predicting various features of the signaler beyond species identity, such as body size, fighting ability, physiological condition, or genetic quality (Bradbury and Vehrencamp, 1998; Fitch 2003). Finally, information on how signalers produce signals in space and time can provide insights about possible intended receivers and potential environmental and social influences on signal production (e.g., Arak and Eiriksson, 1992; Mathevon *et al.*, 1996). Ultimately, statistical descriptions of signals and patterns of signal use are necessary to inform tests of hypotheses about the proximate mechanisms of signal production and perception and the current adaptive functions and past evolutionary histories of signals and signaling behavior.

Our primary objective in this study was to describe the vocalizations and vocal behavior of the Guyanan golden rocket frog, *Anomaloglossus beebei* (Aromobatidae). Similar to most other frogs (reviewed in Gerhardt & 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 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.

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 (KNP; $05^{\circ}10'N$, $59^{\circ}29'W$) (Kok et al. 2006). Our study site consisted of an approximately 100-hectare 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.

B. Acoustic recordings and analyses

Between May and July, 2007, we recorded a total of 916 calls from 60 males (between 6-19 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 towards 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, New Jersey, USA) 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, Connecticut, USA). 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 μ Pa, 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, Maryland, USA). 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 an 8 m radius, a distance greater than any known male territory (Pettitt unpublished data). 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, Illinois, USA); 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) to quantify the following eight spectral and temporal properties for the first pulse and its subsequent interval for each recorded call (Fig. 1.1) — dominant frequency (kHz), pulse duration (ms; onset to offset), pulse interval (ms; offset to onset), pulse rate (pulses/sec; 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 FFT (fast Fourier transform) 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

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. 1.2). 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, Illinois) and we used a significance criterion of $\alpha = 0.05$ for all tests.

1. Data transformations and temperature corrections

We created a dataset 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 sub-divided this dataset into three “call type datasets” 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 dataset using Shapiro-Wilk tests. Of the 27 possible combinations of 9 call properties \times 3 call types, 18 were normal and nine 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 dataset in which normality could be achieved only through a log transformation.

We adjusted call properties correlated with temperature (Table 1.1) 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 dataset ($N = 40$). There

was no difference in air temperature values among the three call type datasets (one-way 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 below were qualitatively unchanged in separate analyses conducted using calls that were transformed to improve normality and corrected for temperature before subdividing the entire dataset into three call type datasets. 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 sec). These standardized values, many of which were highly correlated (see Table 1.2), 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 dataset (advertisement, courtship or aggressive) to which we had subjectively assigned it based on social context. Due to unequal sample sizes among call type datasets, a chance-corrected procedure (Cohen's kappa 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 a Chi-squared test.

4. Relationships with size and condition

For each call type dataset, 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 dataset separately because when we pooled all 60 males into a combined dataset, 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 dataset ($N = 24$). Because these analyses were exploratory (and not confirmatory) in nature, we did not correct for multiple statistical comparisons.

D. Temporal and spatial 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 below. 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 h and 1800 h (e.g. once during 0600-0859, once during 0900-1159, 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 three days with minimal discrepancy (i.e., occasional differences of 1, rarely 2 males).

Separate from our collection of temporal calling data, we measured four characteristics associated with a male's chosen calling site (N = 98 males) as well as the type of call the male was producing immediately prior to data collection. These data were collected between 0600-0900 and 1500-1800 h along the same transect described above and included 78 males producing advertisement calls and 20 males producing aggressive calls. The terrestrial bromeliad leaves from which males call grow out of the center of the plant as stiff, green, up-turned leaves. As the leaves age, they are pushed to the outside by newer leaves and slowly bend downwards until they reach a horizontal angle with the forest floor at their axes. Such older leaves are typically brown and dry and are often surrounded by overhanging green leaves. While the acoustic properties of bromeliads are not well characterized, the propagation of sound can be affected by the

density of vegetation surrounding a signaler (Richards and Wiley 1980). Consequently, the call site characteristics we measured included (i) leaf angle and (ii) leaf color as two measures of the extent of overhanging vegetation. We also measured (iii) height of calling site from the ground because it is also known to influence sound propagation through pattern loss due to boundary reflections (Bradbury and Vehrencamp 1998). Lastly, we measured (iv) bromeliad size because of this characteristic's positive association with the density of vegetation (Richardson 1999). Bromeliad size was categorized as small, medium or large by counting the number of green leaves such that small bromeliads had 3-9 leaves, medium had 10-14 and large had 15 or more leaves. Leaf angle was approximated visually and categorized as 0° , 30° , 45° , 60° or 90° above the horizontal. Leaf color was a binary variable consisting of green or brown and was based on which color made up more than half of the leaf in a subjective visual assessment. Brown leaves are found primarily at the base of the bromeliad and are associated with smaller angles (0° - 30°) above the horizontal. Height of the calling site was measured using a tape measure as the vertical distance (to the nearest 1 cm) between the male's calling site and the ground. We investigated differences in call site characteristics between males producing advertisement calls and those producing aggressive calls using chi-squared and Mann-Whitney *U* tests (two-tailed). We did not include courtship calls in analyses of call type use in time and space because they were too rarely encountered during censuses.

RESULTS

A. Multivariate analyses

1. *Principal components analysis*

The first two components had eigenvalues greater than 1.0 and accounted for 76.7% of the original variation (Table 1.3). 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. 1.3). Principal Component 1, which explained 53.4% 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 1.3). 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 23.3% of the variance and was strongly correlated with dominant frequency, call amplitude, and pulse duration (Table 1.3). 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. 1.3).

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, 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 dB SPL 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. 1.3).

2. Cluster analysis

The CA revealed three main clusters that corresponded almost identically to our initial classifications (Fig. 1.4). 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 Kappa, $k = 0.89$). 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 1.4). The following descriptions and comparisons of call types are based on the calls of males whose call type was verified statistically ($N = 57$).

B. Descriptions and comparisons of call types

All three call types (Fig. 1.5; Table 1.4) are composed of a series of short pulses (25-40 ms each) produced at rates of about 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 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 1.4).

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. 1.5).

Courtship calls had a higher occurrence of non-linear phenomena (89%; 330/370 pulses) than did either advertisement calls (<1%; 11/2679 pulses) or aggressive calls (5%; 18/392 pulses; $\chi^2(2, N = 3241) = 1431.8, p < 0.001$). As illustrated in Figure 1.2, these phenomena included: frequency jumps (10.0% of calls containing non-linear 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 non-linear phenomena. Frequency jumps were equally likely to occur in the pulses at the beginning, middle and end of calls ($\chi^2(2, N = 38) = 3.21, p = 0.20$). The onsets of abrupt frequency changes typically occurred about half way 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 ($\chi^2(2, N = 114) = 29.11, p < 0.001$). 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 (SD; 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%; $\chi^2(2, N = 229) = 26.52, p < 0.001$). While 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 1.5). 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 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 1.5). 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 1.5). Interestingly, aggressive calls were the only call type for which dominant frequency was correlated (negatively) with body size.

D. Temporal and spatial patterns of call type use

Males rarely called prior to sunrise (approximately 0545-0600 h; Pettitt unpublished data), but almost half (45%) of all calls were produced between 0600-0900 h (Fig. 1.6). Males continued to call throughout the day with calling behavior waning after sunset (approximately 1800-1815 h; Pettitt unpublished data). 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. 1.6). 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. 1.6).

All calling males were observed sitting on bromeliad leaves. Individuals were rarely seen on the ground and were never observed to vocalize from such a location. Males producing advertisement calls were more commonly found on green leaves and smaller bromeliads, while males producing aggressive calls were observed more often on brown leaves ($\chi^2 = 7.10, p = 0.008$) and in larger bromeliads ($U = 517.5, p = 0.01$). There were no differences found in leaf angle ($U = 587.0, p = 0.075$) or height from ground ($U = 677.5, p = 0.36$) between males producing the two different call types.

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. While 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 a higher sound pressure level 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. 1.5). 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. 1.5).

Vocal repertoires consisting of three call types produced in similar social contexts have been noted in other aromobatids (e.g., *A. 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 produce 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

while the other was used during territorial advertisement, agonistic interactions, and courtship (Lima & Keller 2003). *Mannophryne trinitatis*, on the other hand, produces one type of advertisement call and at least two types of courtship call, 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 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*. 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. Because 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 & Bee 2007).

Calling activity by male golden rocket frogs peaked between 0600 h 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 some aromabatids and dendrobatids (Stewart & Rand 1992; Juncá 1998). Interestingly, advertisement and aggressive calls had different patterns of usage throughout the day in golden rocket frogs (Fig. 1.6); 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 while aggressive calls were heard approximately 45 minutes later when advertisement calling was waning (Stewart & 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 & 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 & Bastos 2007).

Although some variation in the spatial patterns of calling was observed, all vocalizations recorded and observed as part of the present study were produced by males sitting on bromeliad leaves. Males producing advertisement calls were more commonly found on smaller bromeliads and on green leaves. Males producing aggressive calls were more often on larger bromeliads and on brown leaves and, consequently, were surrounded by a greater amount of overhanging vegetation. While many researchers have examined the general effects of vegetation and height of signalers on sound propagation (e.g., Richards and Wiley 1980; Wells and Schwartz 1982; Mathevon et al. 1996), little is known about how plant size and structure may impact the acoustic properties of calling sites found within bromeliads. Bromeliads are structurally complex (Laessle 1961; Richardson 1999) and calling sites within such plants can be surrounded by dense overhanging vegetation. Vocalizing from bromeliad leaves occurs in other bromeliad breeding anurans. *Phyllodytes luteolus*, for instance, have been observed calling from bromeliad leaf axils while partially submerged in water (Eterovick 1999),

while *Eleutherodactylus melacara* and members of the *Scinax perpusillus* species group have been documented calling from the tips of leaves (Hedges et al. 1992; Alves-Silva and da Silva 2009).

Sound transmission studies to characterize the propagation of frog calls from different positions in vegetation dominated by bromeliads are needed to understand better the decisions male frogs make when selecting calling sites (e.g., Wells and Schwartz, 1982). While the present study did not examine the effects of vegetation on call propagation, we would predict that calls produced by males positioned in the basal region of the plant (i.e., on brown leaves that are often surrounded by overhanging green leaves) would suffer greater attenuation reducing the distance at which the call could be perceived. Likewise, we would predict calls produced by males on larger bromeliads to suffer greater attenuation and degradation simply due to the greater amount of surrounding vegetation. Future investigations addressing the space use patterns of golden rocket frogs will need to examine such acoustic properties as well as other microhabitat characteristics associated with the bromeliad, including water volume, humidity levels, availability of resources, and density of predators. Each of these characteristics may be relevant to a caller's future growth, survival and reproductive success.

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. Furthermore, 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.

Table 1.1: Correlations between air temperature and *A. beebei* advertisement, courtship and aggressive call properties.

	Advertisement Calls (<i>N</i> = 40)	Courtship Calls (<i>N</i> = 12)	Aggressive Calls (<i>N</i> = 8)
Pulse Duration (ms)	-0.374*	-0.362	-0.102
Pulse Interval (ms)	-0.372*	-0.349	0.704
Pulse Rate (pulses/sec)	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

Pearson's correlation coefficients, * $P < 0.05$, ** $P < 0.01$
 Bold values indicate call properties corrected for temperature

Table 1.2: Correlation matrix for call properties using individual means from 60 *A. beebei* males.

	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**

Pearson's correlation coefficients, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Matrix calculated using transformed, temperature-corrected, standardized call properties values ($N = 60$ males).

Identical results were obtained using transformed, temperature-corrected, unstandardized values.

Table 1.3: Factor loadings of the first two principal components using standardized, temperature- corrected input variables. Loading values exceeding 0.6 are highlighted in bold face.

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 dB	0.321	0.769
	-0.330	0.772
Variance (%)	54.57	22.34
Cumulative Variance (%)	54.57	76.91

Table 1.4: Physical characteristics of the vocalizations of adult males of *Anomaloglossus beebei*. Values are expressed as medians, interquartile range (in brackets) and range (in parentheses).

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

Kruskal Wallis test, ** $P < 0.01$, *** $P < 0.001$

Medians values represent the medians of individual means, which were determined by averaging over all calls recorded from an individual. Medians with different letters 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. While Male 28 was classified as producing courtship calls in the DFA, we did not remove this male because he was classified by the CA and our original aural and visual categorizations as producing advertisement calls.

Table 1.5: Pearson correlation coefficients between call properties and mass, SVL and body condition in male *A. beebei*.

	Advertisement Calls			Courtship Calls			Aggressive Calls		
	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/sec)	-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

Adv = advertisement calls (N = 24), Crt = courtship calls (N = 7), Agg = aggressive calls (N = 4)

Pearson's correlation coefficients, * $P < 0.05$, ** $P < 0.01$

Bold values indicate significant associations (see text for explanation)

Figure 1.1: Waveform and spectrogram (see inset) of an *Anomaloglossus bebei* advertisement call showing the spectral call property and five of the temporal call properties quantified in this study. Pulse rate was calculated as $1/\text{pulse period}$ and call rate was calculated as the number of calls delivered per minute.

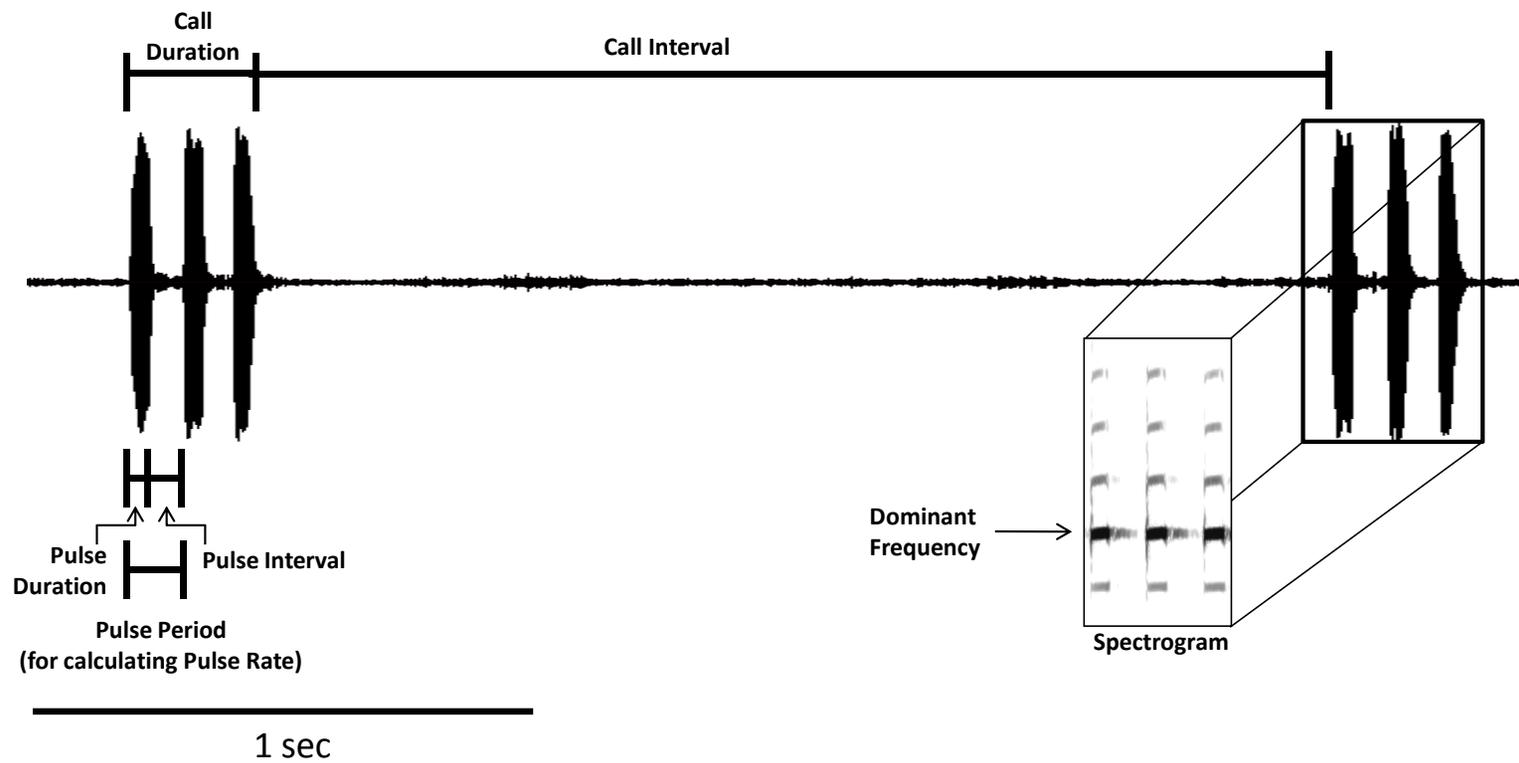


Figure 1.2: Spectrogram and waveform of the three types of nonlinear phenomena found in *Anomaloglossus beebei* vocalizations: (a) frequency jumps, (b) deterministic chaos and (c) subharmonics.

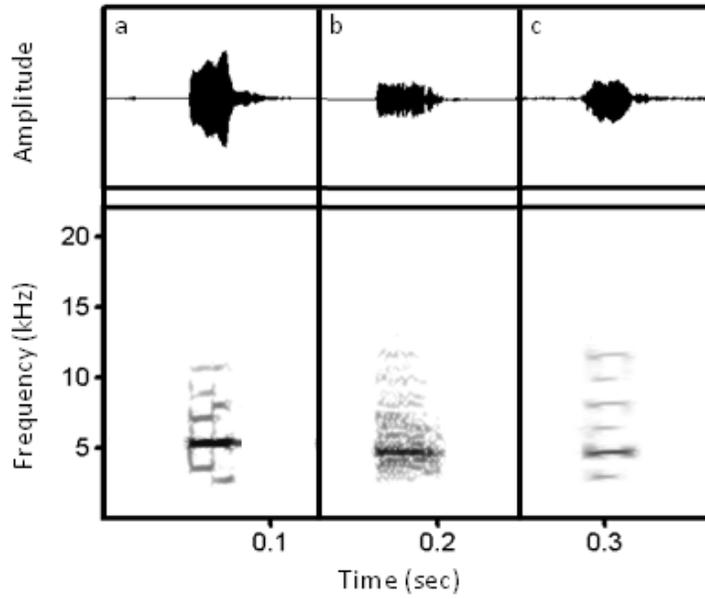


Figure 1.3: Mean scores for the first two principal components for each proposed signal type as determined by subjective analyses (N = 60 males). We initially classified males as producing advertisement (\square), courtship (+) or aggressive (Δ) calls.

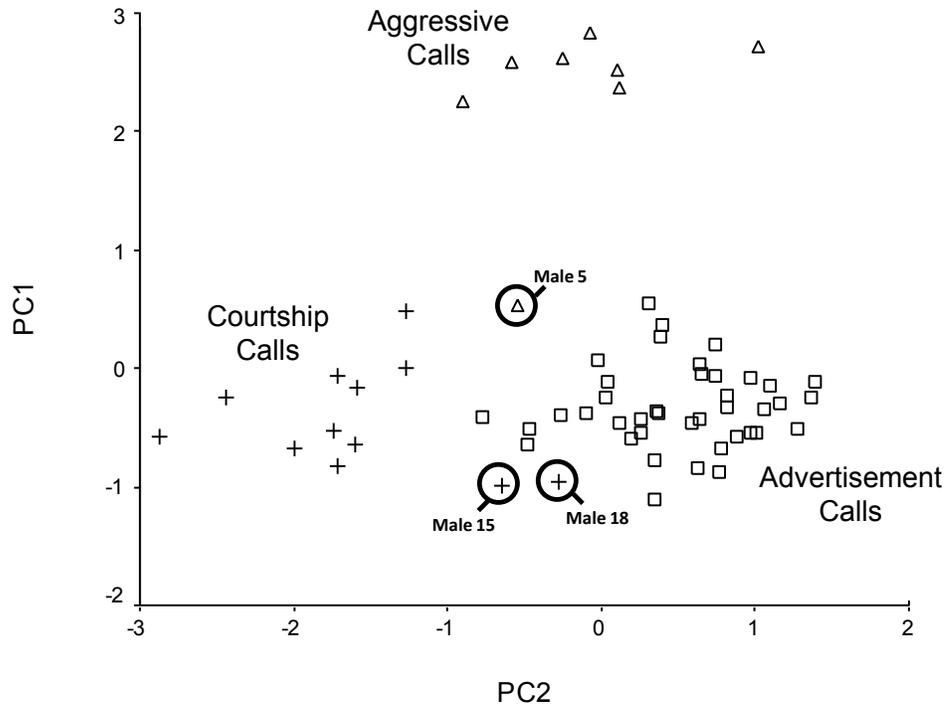


Figure 1.4: A cluster analysis using mean values for call properties from recordings of 60 males. Numbers listed on the left side of the dendrogram correspond to male ID codes. All individuals grouped into one of three clusters: advertisement, courtship, or aggressive calls. Based on our initial visual 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).

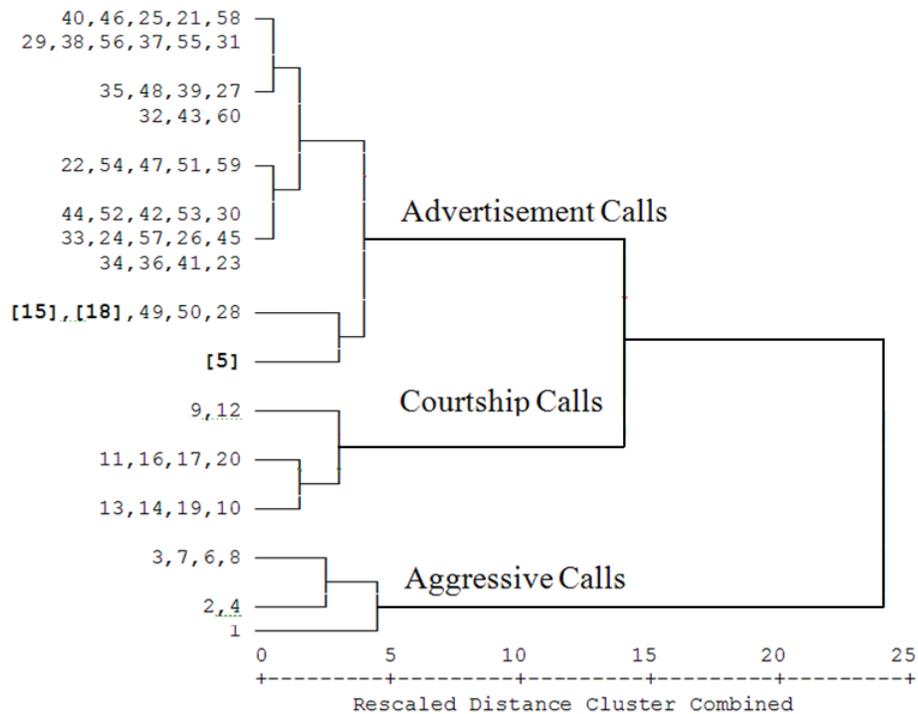


Figure 1.5: Spectrogram (FFT = 512 points, Hanning window, 75% overlap) and waveform of a typical (i) advertisement call, (ii) aggressive call and (iii) courtship call of male *Anomaloglossus beebei* from Kaieteur National Park, Guyana. Air temperature during recordings was 26.1° C, 25.2° C and 23.4° C, respectively.

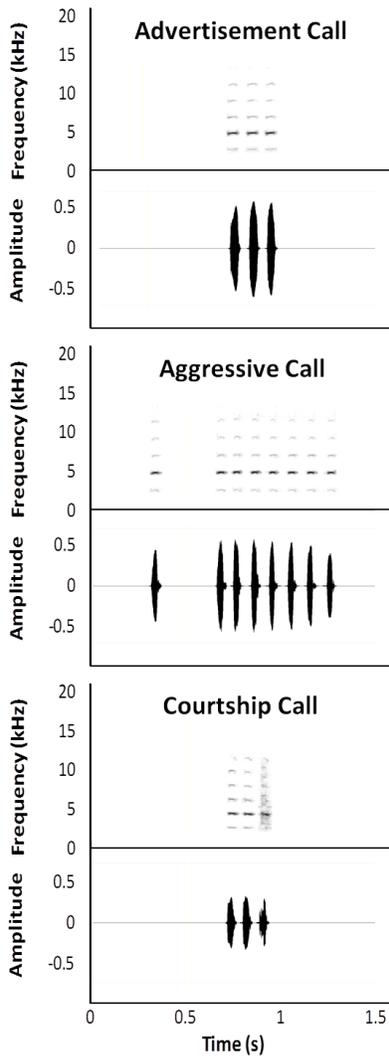
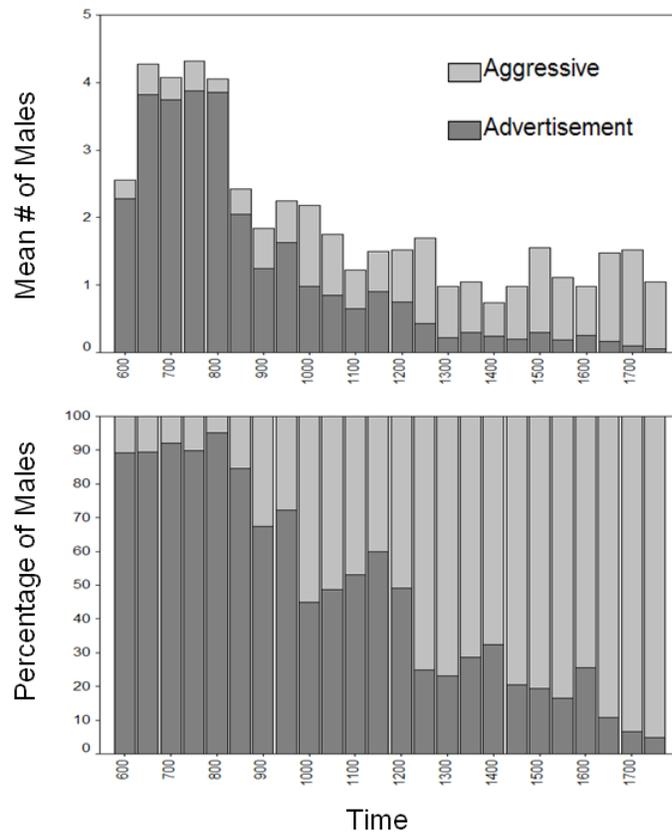


Figure 1.6: The diel pattern of advertisement (dark gray) and aggressive (light gray) calls produced by *Anomaloglossus beebei* during 24 sampling days in July-August 2008, in Kaieteur National Park, Guyana. Each bar represents the mean number (top graph) or the relative proportions (bottom graph) of calling males during each 30-minute period from 0600-1800 h.



**Chapter 2: Advertisement call variation in the golden rocket frog
(*Anomaloglossus beebei*): evidence for individual distinctiveness**

SUMMARY

Individual distinctiveness in acoustic signals can allow receivers to respond appropriately to different individuals. The aim of this research was to examine signal variation and to investigate the relative importance of different acoustic properties for coding individual distinctiveness in the advertisement calls of male golden rocket frogs (*Anomaloglossus beebei*). I examined patterns of within-individual and among-individual variability in 760 advertisement calls of 40 males as well as repeatability in 16 additional males that were recorded twice. I examined eight call properties and all properties exhibited significant among-individual variation and moderate to high repeatability. I employed discriminant function analysis to statistically examine individual distinctiveness. These analyses assigned 79% of calls to the correct individual. Fine temporal properties, including pulse duration, pulse rate and pulse interval, contributed most towards discrimination among individuals. These results indicate that individual male golden rocket frogs can be statistically distinguished by their advertisement calls. I use these findings to evaluate results of previous playback studies in *A. beebei* and to generate testable predictions regarding the potential for specific call properties to function in mate selection and social recognition.

INTRODUCTION

Acoustic communication plays a prominent role in many animal social interactions. Abilities to distinguish among individuals allow receivers to respond adaptively to acoustic signals produced by conspecifics in different social contexts. For example, individual distinctiveness can play an important role in two particular social contexts: (i) mate selection and (ii) social recognition of territorial neighbors (Falls 1982; Vannoni & McElligott 2007; Bee et al. 2010). When male acoustic signals vary consistently, females may use these patterns of variation during mate selection. Investigations into signal variability and female preferences have recognized the importance of examining these patterns of variation due to their association with different types of selection pressures (Gerhardt 1991; Gerhardt & Huber 2002). Specifically, preferences for properties that exhibit high levels of variation tend to be under directional selection and properties that exhibit low levels of variation tend to be under stabilizing or weakly directional selection (Gerhardt 1991). Consequently, estimations of variation can be used to create testable predictions regarding female preferences for particular call properties. Males that defend territories from competing conspecifics can also benefit from acoustic variability if signals are individually distinct. The ability to recognize a familiar neighbor from an adjacent territory would allow a territorial male to conserve time and energy by defending his territory from only intruding, unfamiliar strangers (Eason & Hannon 1994). This form of social recognition is known as neighbor-stranger discrimination and has been demonstrated in a variety of territorial species, including birds (reviewed in Falls

1982; Lambrechts & Dhondt 1995; Stoddard 1996), mammals (e.g. Sousa-Lima et al. 2002), fish (e.g. Myrberg & Riggio 1985), and amphibians (e.g. Davis 1987; Bourne et al. 2001).

Anuran amphibians (frogs and toads) often use acoustic signals in female mate selection and male-male territorial interactions. In particular, species-specific advertisement calls often function to attract conspecific females and repel competing males simultaneously (Wells 1977; Ryan 2001; Gerhardt & Huber 2002). Properties of advertisement calls may be used to assess the caller's species identity, size, condition, genetic or physiological quality, fighting ability and individual identity (reviewed in Gerhardt & Bee 2007). For call properties to function in coding individual distinctiveness, they must exhibit more variation among individuals than within individuals. Thus, an examination of the patterns of variability in the acoustic properties of advertisement calls is the first step towards understanding the role these properties play in identifying individuals.

The golden rocket frog (*Anomaloglossus beebei*) is a territorial dendrobatid that exhibits acoustically mediated mate selection and neighbor-stranger discrimination (Bourne et al. 2001). During courtship and pair formation, male *A. beebei* attract a female to their territory with a loud advertisement call. The call consists of a short series of rapidly repeated pulses (Fig. 2.1; Pettitt et al. 2012). Following egg deposition, males and females provide joint parental care for developing offspring (Bourne et al. 2001). Males call antiphonally or stop moving when they hear advertisement calls of neighbors, while males encountering calls of non-neighbors approach the caller, emit territorial calls

or both (Bourne et al. 2001). The extent to which advertisement call properties code for individual distinctiveness in *A. beebei* has not been investigated. Such investigations are necessary to understand how specific call properties function in different social contexts. The aim of this study was to examine patterns of individual variation and individual distinctiveness to identify the potential functionality of *A. beebei* advertisement call properties. Specifically, we calculated the coefficients of variation among-individuals and within-individuals and estimated individual distinctiveness and repeatability for eight temporal and spectral call properties. Our results reveal the relative importance of each property for coding individuality and generate testable predictions regarding the potential for these properties to function in contexts of mate selection and social recognition.

METHODS

A. Study Site

Golden rocket frogs are endemic to Guyana, South America, where they are found only in Kaieteur National Park (Kok et al. 2006). Our study was conducted in the park along the edge of a 455 m plateau near Kaieteur Falls (05°10'23"N, 59°28'52"W). This frog is a bromeliad specialist (Bourne et al. 2001) and our study site consisted of open glades where giant bromeliads (*Brocchinia micrantha*) and golden rocket frogs were abundant.

B. Acoustic Recordings

We used two different sets of advertisement calls to examine (i) call variability and individual distinctiveness and (ii) estimates of repeatability. To examine variability and distinctiveness, we recorded 760 advertisement calls from 40 males (19 calls/male) between May and July 2007. To estimate repeatabilities of call properties, we recorded 320 advertisement calls from 16 additional males (20 calls/male) between May and August 2009. For each of these 16 males, we recorded 10 calls on each of two different days separated by a median of 2.5 days (range: 1 – 29 days apart). For all recordings we used a handheld Sennheiser ME66 directional microphone (Sennheiser Electronic Corporation, Old Lyme, Connecticut, USA) and either a Marantz PMD620 or PMD670 solid-state recorder (44.1 kHz sample rate, 16-bit resolution; Marantz America, Inc., Mahwah, New Jersey, USA). Throughout each recording session, the tip of the microphone was held $1 \text{ m} \pm 10 \text{ cm}$ from a calling male sitting on the leaf of a bromeliad. We captured recorded individuals when possible and clipped a unique number of toes for identification (24 of 40 males recorded in 2007; 16 of 16 males recorded in 2009). Frogs were released immediately after marking and measuring at the capture site. To remove the risk of resampling an unmarked male, we never recorded more than one male within a 200 m^2 area, an area greater than any known male territory. Immediately following each recording, we measured air temperature at the site from which the male had been calling using an Oakton digital thermohygrometer (Oakton Instruments, Vernon Hills, Illinois, USA). These temperatures ranged between 22.0° C and 29.6° C with a mean of 24.7° C . Recordings were made in the mornings from 0600-0900 hours.

C. Acoustic Analyses

We used Avisoft-SASLab Pro (Avisoft Bioacoustics, Berlin) to measure or calculate eight temporal and spectral properties of each of the 1080 recorded calls (see Fig. 2.1 for schematic details). We measured the following temporal properties to the nearest 0.1 ms: call duration (onset of first pulse to offset of last pulse), call interval (offset of last pulse of one call to onset of first pulse of next consecutive call), pulse duration (onset to offset) and pulse interval (offset of one pulse to onset of next consecutive pulse). We calculated instantaneous call rate (calls per minute) by taking the reciprocal of the instantaneous call period, which was the sum of a call's duration and subsequent interval. Similarly, we calculated instantaneous pulse rate (pulses per second) by taking the reciprocal of the instantaneous pulse period which was the sum of a pulse's duration and subsequent interval. We counted the number of pulses per call and, using Avisoft's spectrogram function (512 pt. FFT, Hanning window), we measured dominant frequency to the nearest 0.01 kHz. In all cases, the dominant frequency was the second harmonic. Call properties associated with pulses characteristics (e.g., pulse duration, pulse interval, pulse rate and dominant frequency) were measured or calculated for each pulse in each call.

D. Statistical Analyses

Because temperature can affect anuran call properties (Gerhardt & Huber 2002), we temperature-corrected all properties significantly correlated with temperature by adjusting their values to 24° C (near the mean temperature of our recordings) following Platz & Forrester (1988); see Supplementary Material for additional details. Temperature

corrected values of these properties were used in all subsequent statistical analyses. We transformed four call properties to achieve normality (call duration, log; call rate, inverse; pulse interval, inverse; dominant frequency, squared). We report back-transformed, temperature-corrected descriptive statistics and coefficients of variation for all call properties. All analyses were computed using either SPSS v12.0 (SPSS, Inc., Chicago, IL, U.S.A.) or Matlab v7.6 (Mathworks, Natick, MA, U.S.A.).

Assessments of individual distinctiveness rely on the relationship between among-individual and within-individual variation. All *A. beebei* calls analyzed in this study were multi-pulse calls with most calls consisting of three or four pulses (Fig. 2.1). Studies of variation in the pulses of multi-pulse anuran calls often analyze a small subset of pulses per call (Gerhardt & Doherty 1988; Castellano & Giacoma 1998; Bee & Gerhardt 2001a). Because analyses using only a subset of pulses reduce or eliminate among-pulse variability within an individual's calls and thus have the potential to overestimate among-individual variability, we investigated this possibility using our call variability dataset. We performed model II ANOVAs on all call properties related to pulses (i.e., dominant frequency, pulse duration, pulse interval, and pulse rate) to examine the main effects of male ($N = 40$ males), call ($N = 19$ calls per male) and pulse ($N = 3$ pulses per call per male) using the first three pulses of each call. Values of pulse rate and pulse interval for the third pulse of calls containing only three pulses were treated as missing data. We also conducted model II ANOVAs on these same properties using only the first pulse and compared the analyses of one pulse and three pulses using a variance ratio F test (Sokal & Rohlf 1981). Because results of the variance ratio tests showed that

analyses of the first pulse accurately describes the relationship between among-individual and within-individual variation (see Results, Table 2.1), we conducted all subsequent analyses using the first pulse of each call.

We investigated the magnitude of variability in call properties both within-individuals and among-individuals by first calculating individual means (\bar{X}_w) and standard deviations (SD_w ; based on averaging 19 calls per male) as well as a grand mean and standard deviation (based on averaging the 40 individual means) for each of the eight call properties. Using \bar{X}_w and SD_w for each property, we calculated a within-individual coefficient of variation ($CV_w = 100\% \times SD_w / \bar{X}_w$). Similarly, using the grand mean and standard deviation, we calculated an among-individual coefficient of variation ($CV_a = 100\% \times \text{grand } SD / \text{grand } \bar{X}$) for each call property. We determined the ratio of among-individual and within-individual variation as CV_a / CV_w . Following Gerhardt (1991), we identified call properties with a CV_w of less than 5% as static, greater than 12% as dynamic, and between 5% and 12% as intermediate. We also examined variability among males in terms of the difference between the largest and smallest individual means divided by the grand mean. While we report median and interquartile range for the number of pulses per call (a discrete variable), we calculated variability as described above.

We used model II ANOVAs to describe the extent to which each call property varied significantly more among than within individuals. We report the effect size for each ANOVA (partial η^2) as these values describe the proportion of the total variability of a call property that is attributable to among-individual differences. Consequently, the

effect sizes provide additional evidence of the extent to which call properties vary significantly more among individuals than within individuals.

To estimate the individual distinctiveness of *A. beebei* advertisement calls and to determine which call properties contributed most to coding individual distinctiveness, we conducted a discriminant functional analysis (DFA). DFA is a powerful tool for identifying how acoustic properties contribute to discrimination among individuals (e.g., Bee et al. 2001; Gasser et al. 2009; Feng et al. 2009). In this analysis, the canonical discriminant functions generated are used to classify calls as belonging to a particular individual (Sokal & Rohlf 1981). Classification success serves as a measure of how well the discriminant functions distinguish among individuals. We assessed the statistical significance of correct classifications following Titus et al. (1984). Because many of the call properties examined were highly correlated (Pettitt et al. 2012), we used principal components analysis (PCA) to create a set of eight orthogonal predictor variables. We used the corresponding principal component scores as input variables for the DFA. We measured classification success using a “leave-one-out” cross-validation procedure in which each call was assigned to a particular individual based on the discriminant functions generated for the dataset containing the n-1 remaining calls.

We estimated the repeatability of each advertisement call property using a separate set of calls consisting of two recordings from each of 16 males. Repeatability is defined as the fraction of phenotypic variation that is due to differences among individuals (Falconer 1981) and allows for the measurement of among-individual variation while taking within-individual variation into account (Boake 1989). We used

the intraclass correlation coefficient as our measure of repeatability (after Sokal & Rohlf 1981; Lessells & Boag 1987). We also calculated the percent change in the mean value of each call property between recordings.

RESULTS

Regardless of whether we analyzed the first three pulses or only the first pulse of the multi-pulse calls, all properties varied significantly among males (Table 2.1). Analyses involving three pulses showed that only dominant frequency varied among calls and all properties varied among pulses (Table 2.1). Analyses of one pulse and three pulses both classified the same properties as static and dynamic; differences in mean CV_w between the two analyses ranged from 0.1 – 0.9% and differences in CV_a ranged from 0.2 – 1.8%. Results of Model II ANOVAs and the variance ratio tests showed that both analyses produced similar estimates of among-individual variability (Table 2.1). These findings indicate that analyses involving only the first pulse of a call accurately estimated the magnitude of among-individual variability in *A. beebei* advertisement calls.

Descriptive statistics and variability for all call properties are given in Table 2.2. Based on their CV_w , we categorized dominant frequency and pulse rate as static properties; call interval and call rate as dynamic properties; and pulse interval, pulse duration, call duration and number of pulses per call as intermediate properties. Variation among individuals was significantly greater than within individuals for all properties (Table 2.2). Call properties that varied more among individuals also tended to

vary more within individuals (e.g., call duration, call interval, call rate; Table 2.2). The one spectral property examined, dominant frequency, exhibited the least amount of variation both among-individuals and within-individuals. Nevertheless, dominant frequency as well as pulse rate, the two properties classified as static, exhibited higher CV_a/CV_w ratios (3.2 and 2.0, respectively) compared to both dynamic properties (range: 0.8–1.3) and three of the four intermediate properties (range: 1.3–1.8; excluding pulse interval with 2.1; Table 2.2).

Model II ANOVAs based on the first pulse of advertisement calls revealed that all eight call properties exhibited statistically significant among-individual variability (Table 2.2). Moreover, the effect sizes associated with these ANOVAs present a similar trend apparent in the coefficients of variation described above. Static properties with higher CV_a/CV_w ratios were also associated with higher effect sizes, while dynamic properties with lower CV_a/CV_w ratios were associated with lower effect sizes (Table 2.2).

In Tables 2.3 and 2.4 respectively, we report PCA factor loadings and DFA standardized canonical discriminant function coefficients from the analysis of all 760 calls ($N = 40$ males). These DFA coefficients suggest the relative importance of each PCA factor in the composition of the canonical discriminant function score. Our initial PCA extracted eight factors with the first five factors explaining 98% of the variance in the call properties examined. Each of these five factors explained similar amounts of variation (between 10% and 30%; Table 2.3). Results of the DFA using the PCA factor scores as input variables showed that 78.9% of advertisement calls were correctly classified as belonging to the male that produced them ($N = 40$). This level of correct

classification is significantly greater than expected by chance (1/40 or 2.5%; Cohen's Kappa = 0.784; 95% confidence interval = 0.753-0.815; $Z = 134.97$, $p < 0.01$).

Taken together, results from our PCA and DFA indicate that variables associated with pulse temporal structure contribute most to individual distinctiveness (Fig. 2.2). For brevity's sake we consider the first three discriminant functions (DF), which together explained 85.2% of the variance in PCA factor scores. The first DF explained 44.0% of the variation in PCA scores and PCA factors 4 and 6 contributed most to this function's discrimination ability (Table 2.4). PCA factors 4 and 6 loaded heavily on pulse temporal structure including pulse duration, pulse interval, and pulse rate (Fig. 2.2a; Table 2.3). The second DF explained 28.9% of the variation in PCA scores and PCA factor 5 contributed most to its discrimination ability (Table 2.4). PCA factor 5 loaded most heavily on dominant frequency (Fig. 2.2a; Table 2.3). The third DF explained 12.3% of variation and PCA factors 2, 3 and 7 contributed most to its discrimination ability (Table 2.4). These three PCA factors loaded most heavily on both pulse and call temporal structure (i.e., pulse rate, pulse duration, call duration, number of pulses per call and call interval; Fig. 2.2a; Table 2.4). The remaining DFs each explained less than 8% of the variation in PCA scores; most explained less than 5% (Table 2.4). As an aside, we would note that we obtained qualitatively similar results when we used 'original' (temperature-corrected and transformed) call property values instead of PCA scores as input variables in the DFA (Fig. 2.2b).

Call analyses of males that were recorded twice revealed moderate to high repeatability estimates for all call properties examined (Table 2.5). Pulse duration had

the highest repeatability (intraclass $r = 0.65$) while call duration had the lowest (intraclass $r = 0.46$). The highest percent change between recordings occurred in the two properties classified as dynamic, call rate and call interval (17.3% and 20.2%, respectively), while the lowest change was in the static property of dominant frequency (3.2%; Table 2.5). Because these findings suggested a possible association between repeatability and within-individual variation, we examined this relationship across our eight call properties. We found a sizeable, but not significant, negative correlation between estimates of repeatability and CV_w for the eight call properties investigated (Spearman rank correlation, $N = 8$, $r_s = -0.479$, $p = 0.23$).

DISCUSSION

Using two-choice playback experiments, Bourne et al. (2001) showed that female *A. beebei*, in the context of mate selection, and male *A. beebei*, in the context of social recognition between territorial neighbors, *behaviorally* discriminate between males based solely on individual differences in vocalizations. Results from the present study extend the findings of Bourne et al. (2001) by demonstrating that the calling behavior of *A. beebei* differs more among individuals than within individuals and that individuals can be *statistically* distinguished by their advertisement calls. While our results indicated that all acoustic properties examined potentially function as cues for identifying individuals by their calls, properties associated with fine temporal structure contributed most to individual distinctiveness.

A. Individual Call Variation and Mate Selection

All advertisement call properties examined in this study exhibited greater variability among individuals than within individuals and levels of variation among and within individuals showed similar patterns. Dynamic call properties exhibited more variation among individuals as well as within individuals, while static call properties exhibited less variation both among and within individuals (Table 2.2). Broadly similar patterns of variation have been reported for other anurans (Bee et al. 2001; Bee & Gerhardt 2001a; Briggs 2010; Gasser et al. 2009; Gerhardt et al. 1996).

Following Gerhardt (1991), the least variable properties within males, pulse rate and dominant frequency, were classified as static. These findings are consistent with previous studies in other anurans and insects (reviewed in Gerhardt & Huber 2002). Static properties tend to be under stabilizing or weakly directional selection with females often preferring values at or near population means. Such properties are suggested to play an important role in species recognition. Pulse rate, in particular, is an important species recognition cue for many anurans, especially in closely related species that live in sympatry (Loftus-Hills & Littlejohn 1971; Schwartz 1987; Schul & Bush 2002; Straughan 1975). While dominant frequency is also commonly classified as static and is proposed to be used in species recognition, patterns of preferences for this call property are more variable than those based on pulse rate (Gerhardt 1991). This variation may stem from the fact that both body size and temperature can affect frequency preferences (Gerhardt & Huber 2002).

Kaiei's rocket frog, *Anomaloglossus kaiei*, is closely related to *A. beebei* and the two species live sympatrically in Kaieteur National Park. Kaiei's rocket frog produces a call that is similar to *A. beebei*'s short, pulsed advertisement call, but has a slower pulse rate (average = 7.21 pulses/sec compared to *A. beebei*'s 11.3 pulses/sec) and a lower dominant frequency (average = 4.85 kHz compared to *A. beebei*'s 5.4 kHz; Kok et al. 2006, Pettitt et al. 2012). We predict that these two call properties may be under multiple selection pressures simultaneously. Both properties were classified as static and are expected to be under stabilizing selection (Gerhardt 1991). However, given that selection should favor signal divergence among closely related sympatric species to maintain species integrity (Noor 1999), we also might expect *A. beebei* females to exhibit weakly directional or directional preferences for faster pulse rates and higher frequencies. Analyses of the between-species overlap in these call properties may provide insights into the significance of these different selection pressures. While a number of studies have found an inverse relationship between dominant frequency and male body size in anurans (reviewed in Gerhardt & Huber 2002), a previous study with *A. beebei* found no correlation between snout-vent length (SVL) or mass and dominant frequency, suggesting that this call property does not provide receptive females or territorial competitors with reliable information on the size of callers (Pettitt et al. 2012). Thus, dominant frequency may not play a role in assessing individual size, but may be useful in species recognition in *A. beebei*. Future playback tests could identify patterns of female preferences for this call property to determine the potential role frequency plays in species recognition in areas where both *A. beebei* and *A. kaiei* are found.

Call rate and call interval, two intrinsically related properties, exhibited the highest amounts of within-male variation and were classified as dynamic properties. Unlike static properties, dynamic call properties are expected to be under strong directional selection. Preferences for higher call rates have been found consistently in female playback experiments (reviewed in Ryan & Keddy-Hector 1992; Gerhardt & Huber 2002), including a study of *A. beebei*, (Bourne et al. 2001). Because signaling at high rates is strongly correlated with increased metabolic costs for many anurans (Wells 2001) and call rate in *A. beebei* is positively correlated with male body condition (i.e., size-independent body mass; Pettitt et al. 2012), this call property has potential to reliably indicate a male's competitive and resource-acquiring abilities. Under such a scenario, females that exhibit a directional preference for faster call rates may receive indirect benefits (e.g., good genes) or direct benefits (e.g., better oviposition sites), or both. For example, female spadefoot frogs (*Spea multiplicata*) that were mated to males with higher-than-average calling rates had higher fertilization success than females that were mated to males with lower-than-average calling rates (Pfennig 2000). Future preference tests using artificial stimuli could be used to assess quantitatively the strength of female preference for this call property across the natural range of variation found in *A. beebei* advertisement calls.

The remaining four call properties (pulse interval, pulse duration, number of pulses per call, and call duration) were classified as intermediate. Given that static and dynamic categories represent two ends of a continuum of variability (Gerhardt 1991), call properties with values of CV_w that fall just above 5% or below 12% may follow the

patterns of female preferences associated with static or dynamic classifications, respectively. For example, in *A. beebei*, pulse interval, which is reciprocally-related to pulse rate (a static property), had a relatively low CV_w (5.4%) and thus, we would expect it to be under stabilizing selection with females preferring values at or near the population mean. It is important to note, however, that preferences for pulse interval, like pulse rate, may also be under weakly directional or directional selection for shorter intervals to maintain divergence in advertisement calls between *A. beebei* and *A. kaiei* (see above). Call duration, on the other hand, with a CV_w of 10.5%, may more likely be under directional selection with females preferring longer calls. In other anurans, estimates of CV_w for call duration vary considerably across species. This call property has been classified as static (Gerhardt 1991; Friedl & Klump 2002; Bee et al. 2010), dynamic (Gerhardt 1991; Castellano & Giacoma 1998; Howard & Young 1998) and intermediate (Bee et al. 2001; Pröhl 2003; this study). Gerhardt (1991) suggests that this large range of within-individual variability in call duration across species is related to the temporal structure of a species' vocalization, with more variability seen in species with distinctly pulsatile calls (pulse rates of 100 pulses/s or less and distinct periods of silence between pulses) compared to unpulsed or very rapidly pulsed calls. Advertisement calls of *A. beebei* appear to follow this pattern, as calls consist of distinct pulses (pulse rate = 11.3 pulses/s) and call duration exhibits a relatively high average CV_w (10.5%).

Estimating the repeatability of behaviors can also inform investigations of individual variation and is necessary for assessing patterns of selection (Boake 1989). Because repeatability is considered an estimate of the upper limit of heritability of a

property (Falconer 1981), our moderate to high (0.46-0.65; Table 2.5) repeatability estimates for *A. beebei* call properties suggests that genetic differences among males could account for up to about half of the variation exhibited by these properties. These results also suggest the potential for *A. beebei* call properties to respond to selection pressures. Low estimates of repeatability are expected to be associated with high within-individual variation and this relationship has been found in the call properties of some anurans (e.g., *Bufo americanus*, Howard & Young 1998; *Physalaemus eneseftae*, Tárano 2001; *Rana catesbeiana*, Bee & Gerhardt 2001a). This relationship was less evident in *A. beebei* as well as in some other species (e.g., *Hyla versicolor*, Gerhardt 1991; *O. pumilio*, Pröhl 2003). Our analysis revealed a sizeable, although not significant, negative correlation between these two variables.

B. Individual Distinctiveness and Social Recognition

The DFA revealed that male *A. beebei* advertisement calls are highly individually distinctive, with 79% of calls correctly assigned to individual callers using a sample of 40 males. Analyses of smaller (more biologically relevant) group sizes resulted in even better classification (see Supplementary Material). Finding individual distinctiveness in animal vocalizations is not surprising (Bee et al. 2010; Charlton et al. 2011; Reers et al. 2011). Compared to other anurans, our classification results were higher overall than those found in *Odorrana tormota* (54.6%; Feng et al. 2009) and *Allobates femoralis* (20-93%; Gasser et al. 2009), and were similar to those found for *R. catesbeiana* (72-92%; Bee & Gerhardt 2001a), *R. clamitans* (52-100%; Bee et al. 2001), and *P. maculata* (55-

100%; Bee et al. 2010). Studies examining individual distinctiveness in other taxonomic groups have reported similar magnitudes of classification success, including birds (70.6–97.5%; Lein 2008; Clark & Leung 2011; Reers et al. 2011) and mammals (56–87.7%; Blumstein & Muños 2005; Grilliam et al. 2008; Charlton et al. 2011). It should be noted, however, that some variation among studies could result from differences in sample size (Bee et al. 2001; Beecher 1989).

Pulse temporal structure contributed most to individual distinctiveness in *A. beebei*, followed by spectral structure and gross temporal structure. Together with our analyses of variability (e.g., CV_a/CV_w and model II ANOVAs), these results indicate that all of the acoustic properties examined here potentially function as cues for identifying individuals by their calls. Identifying individuals in the context of territorial neighbor-stranger discrimination has been previously demonstrated in both *A. beebei* (Bourne et al. 2001) and *R. catesbeiana* (Davis 1987) using field playback experiments; however, statistical analyses of call variation suggest that the call properties coding individual distinctiveness differ between these two species. In *R. catesbeiana*, fundamental frequency (≈ 100 Hz) and dominant frequency (≈ 200 Hz), which were strongly negatively correlated with male size, contributed the most toward individual distinctiveness (Bee & Gerhardt 2001a). Bee and Gerhardt (2001a) argued that size-related constraints on spectral properties give rise to individually distinct calls in *R. catesbeiana* and they showed that receivers use this cue in discriminating between familiar and unfamiliar calls (Bee & Gerhardt 2001b). It is unlikely, however, that this same recognition mechanism is at play in *A. beebei*. There was no relationship between dominant frequency and male

size (Pettitt et al. 2012) and dominant frequency does not appear to contribute as much as other properties (e.g., pulse temporal properties) to individual distinctiveness.

Additionally, *A. beebei* produce calls with high frequencies (average = 5.4 kHz) that are likely encoded by the basilar papilla, one of two auditory sensory organs in the anuran inner ear that is sensitive to frequencies above 1000 Hz. Because frequency discrimination by the basilar papilla is likely dependent on sound intensity (Zakon & Wilczynski 1988) and the high frequency calls of *A. beebei* may experience high rates of attenuation, this spectral call property may be a less reliable option for coding individual distinctiveness. Instead, our results suggest the hypothesis that individual distinctiveness in *A. beebei* may be based on fine temporal call properties, including pulse duration, pulse interval and pulse rate. All three properties showed low variation within individuals, highly significant among individual variability, and high estimates of repeatability. Both pulse rate and pulse interval exhibited high CV_a/CV_w ratios and pulse rate was the only call property correlated with SVL (Pettitt et al. 2012). Future playback studies using synthetic calls that vary in different properties could examine the potential role these call properties play in discrimination tasks (e.g., Bee & Gerhardt 2001c).

C. Conservation Applications

Recent studies have proposed and tested new bioacoustic tools that use the individual distinctiveness of animal vocalizations to census threatened wildlife populations (e.g., Terry et al. 2005; Tripp & Otter 2006). It is now well established that many anuran species and populations are threatened and in decline (Semlitsch 2003; Lannoo 2005). As

of 2008, *A. beebei* was listed as vulnerable under the IUCN Red List of Threatened Species because it is only known from one location, Kaieteur National Park, and its area of occupancy within the park is small ($< 20 \text{ km}^2$; Kok 2008). Consequently, the development of non-invasive methods that could be used to monitor populations within this area would be beneficial to this species' conservation. Future studies should investigate the potential for such tools (e.g., multi-microphone arrays; Jones & Ratnam 2009) in the conservation of *A. beebei* and many other threatened and endangered anuran species.

Table 2.1: Results of model II ANOVAs examining among-male variability based on multiple pulses and the first pulse and results of F tests of the ratio of the variance attributable to the effect of Male.

Call Property	Source	Multiple pulses		First pulse only		Variance ratio	
		F^*	P	$F_{39,720}$	P	$F_{39,39}$	P
Pulse Duration	Male	79.6	<0.01	22.3	<0.01	1.28	0.22
	Call	1.2	0.22				
	Pulse	67.5	<0.01				
Pulse Interval	Male	131.2	<0.01	69.1	<0.01	1.00	0.50
	Call	0.6	0.90				
	Pulse	85.8	<0.01				
Pulse Rate	Male	50.5	<0.01	54.4	<0.01	1.38	0.16
	Call	0.5	0.97				
	Pulse	106.5	<0.01				
Dominant Frequency	Male	539.1	<0.01	158.6	<0.01	1.01	0.49
	Call	4.2	<0.01				
	Pulse	482.5	<0.01				

*Degrees of freedom: Male (39, 2201), Call (18, 2201), Pulse (2, 2201) for Pulse duration, Frequency and Male (39, 1707), Call (18, 1707), Pulse (2, 1707) for Pulse interval, Pulse rate

Table 2.2: Means (or median where indicated) and standard deviations (SD, or inter-quartile range (IQR) where indicated) and measures of within-individual and among-individual variability for eight acoustic properties standardized to a common water temperature of 24° C. Analyses are based on the first pulse of 760 advertisement calls from 40 *Anomaloglossus beebei* males (19 calls/male). Horizontal lines distinguish properties classified as static, intermediate and dynamic (from top to bottom).

Call Property	Grand Mean or Median	SD or IQR	Mean CV _w (range)	Average range of variation within males (% of means)	CV _a	CV _a /CV _w ratio	Max among-male difference (% of grand mean)	Model II ANOVA F _{39,720}	Partial η ²
Dominant Frequency (kHz)	5.4	0.2	1.2 (0.0-3.2)	4.0	3.8	3.2	19.5	158.6*	0.90
Pulse Rate (pulses/sec)	11.3	0.8	3.4 (1.5-10.2)	12.6	6.7	2.0	28.0	54.4*	0.75
Pulse Interval (ms)	54.8	6.3	5.4 (3.4-10.7)	19.8	11.6	2.1	58.1	69.1*	0.76
Pulse Duration (ms)	35.1	3.3	7.3 (2.5-28.1)	28.3	9.4	1.3	43.3	22.3*	0.55
Pulses Per Call	3.0 [†]	1.0 [‡]	7.7 (0.0-20.7)	22.6	13.9	1.8	53.8	38.7*	0.68
Call Duration (ms)	243	43	10.5 (1.4-26.5)	34.9	17.8	1.7	70.7	35.6*	0.66
Call Rate (calls/min)	27.0	4.9	14.2 (3.1-31.2)	57.4	18.2	1.3	80.4	6.9*	0.27
Call Interval (ms)	2142	377	22.1 (5.2-66.1)	92.3	17.6	0.8	71.9	6.6*	0.27

[†] Median, [‡] Interquartile Range
**P* < 0.01

Table 2.3: Results from a principal components analysis showing the factor loadings for all eight factors.

Call Property	PCA Factor							
	1	2	3	4	5	6	7	8
Pulses Per Call	0.675	0.629	-0.301	0.091	0.183	0.025	-0.126	-0.017
Call Duration	0.696	0.488	-0.481	0.111	0.130	-0.034	0.121	0.024
Call Interval	0.756	-0.426	0.447	0.172	-0.093	0.014	-0.028	0.087
Call Rate *	0.804	-0.383	0.403	0.176	-0.070	0.002	0.032	-0.087
Pulse Duration	-0.140	-0.443	-0.569	0.620	-0.196	0.193	-0.004	-0.002
Pulse Interval *	-0.346	0.345	0.250	0.814	-0.029	-0.187	-0.006	0.000
Pulse Rate	-0.188	0.706	0.618	0.131	0.119	0.228	0.032	0.003
Dominant Frequency	-0.120	-0.472	0.011	0.147	0.861	0.008	0.003	0.001
Eigenvalue	2.346	1.999	1.453	1.168	0.859	0.127	0.034	0.016
Variance (%)	29.3	25.0	18.2	14.6	10.7	1.6	0.4	0.2
Cumulative % of variance	29.3	54.3	72.5	87.1	97.8	99.4	99.8	100

Loadings within 30% of the largest value for each factor are highlighted in boldface type.

* Call rate and call interval, two reciprocally related call properties, exhibit identical signs because call rate was transformed using an inverse transformation. Likewise, pulse rate and pulse interval exhibit identical signs because pulse interval was transformed using an inverse transformation.

Table 2.4: Standardized canonical discriminant function coefficients showing the relative importance of each PCA factor score (on the original eight call properties; see Table 3) in the composition of the discriminant functions.

PCA Factor	Discriminant Function							
	1	2	3	4	5	6	7	8
Factor 1	0.251	-0.356	-0.003	-0.810	0.562	0.007	0.448	-0.001
Factor 2	0.120	-0.762	0.756	0.242	0.519	0.140	-0.213	0.066
Factor 3	0.049	0.500	0.837	0.333	-0.338	0.443	0.434	-0.017
Factor 4	-1.093	0.197	0.144	0.585	0.294	-0.550	0.263	-0.080
Factor 5	0.360	1.169	0.070	-0.028	0.266	0.011	-0.083	0.020
Factor 6	1.393	-0.183	-0.032	0.199	-0.029	-0.150	0.069	-0.026
Factor 7	-0.054	-0.122	-0.856	0.496	0.338	0.377	0.103	-0.004
Factor 8	-0.015	0.024	-0.110	0.100	-0.088	-0.123	0.118	0.979
Eigenvalue	15.976	10.494	4.475	2.576	1.724	0.745	0.227	0.124
Variance (%)	44.0	28.9	12.3	7.1	4.7	2.0	0.6	0.3
Cumulative % of variance	44.0	72.8	85.2	92.2	97.0	99.0	99.7	100.0

Coefficients within 30% of the largest value for each function are highlighted in boldface type.
DFA, discriminant function analysis; PCA, principal components analysis

Table 2.5: Call property repeatabilities of 16 *Anomaloglossus beebei* males recorded twice. Horizontal lines distinguish properties classified as static, intermediate and dynamic (from top to bottom).

Call Property	Intraclass r	Average change (%)
Dominant Frequency (kHz)	0.55	3.2 (0-7.7)
Pulse Rate (pulses/sec)	0.54	6.3 (1.4-13.1)
Pulse Interval (ms)	0.60	9.6 (0.7-22.1)
Pulse Duration (ms)	0.65	5.1 (0.9-13.5)
Pulses Per Call	0.49	8.9 (0-32.3)
Call Duration (ms)	0.46	11.9 (0.8-30.3)
Call Rate (calls/min)	0.55	17.3 (1.7-31.6)
Call Interval (ms)	0.48	20.2 (1.9-36.2)

Figure 2.1: Analysis of temporal and spectral call properties. Plots (a) - (c) depict waveforms of (a) 10 consecutive calls from one male, (b) a close-up showing two calls from the same male, and (c) a close-up showing two pulses from the call indicated in plot (b). Horizontal bars indicate different time scales. (d) Spectrogram showing the harmonic structure and dominant frequency (second harmonic) of a three pulse call.

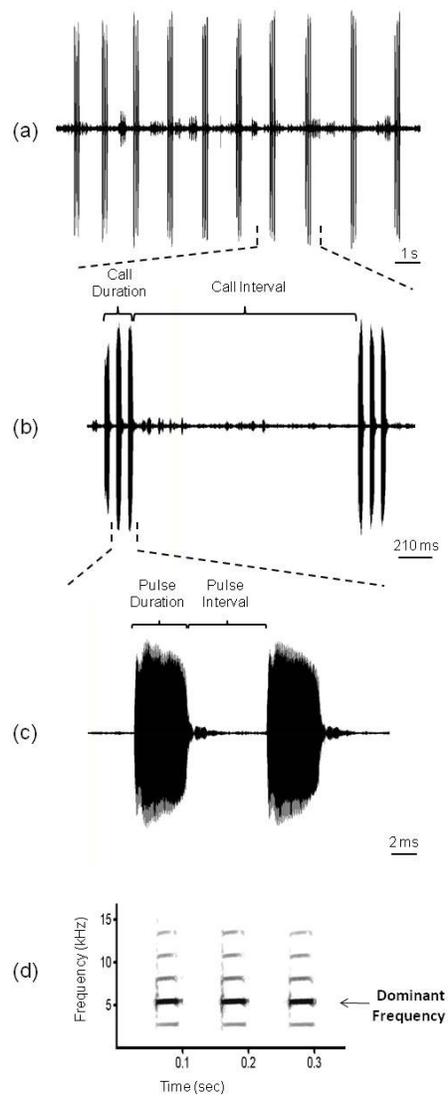
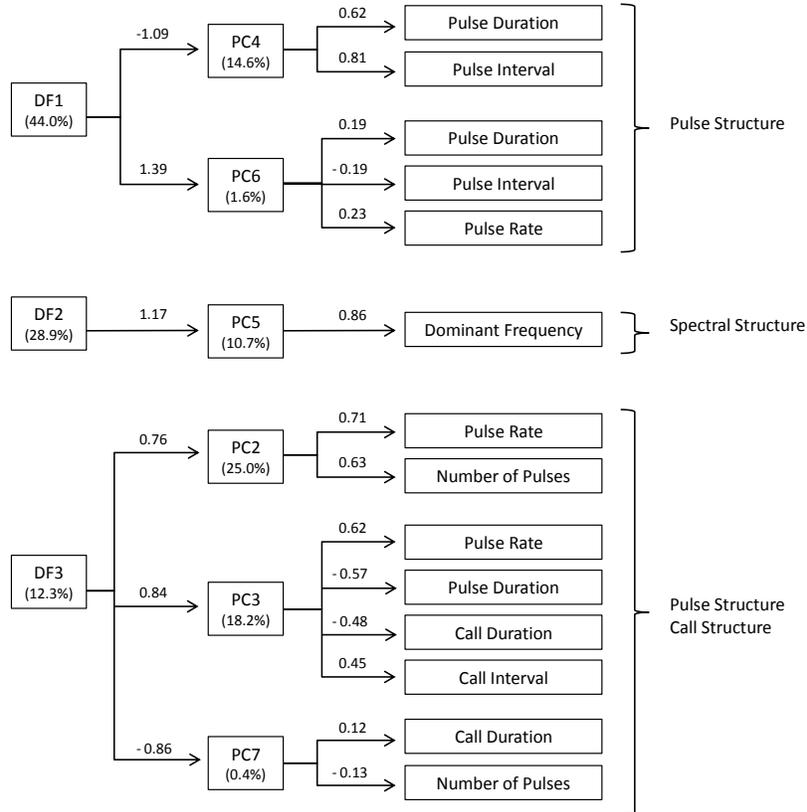


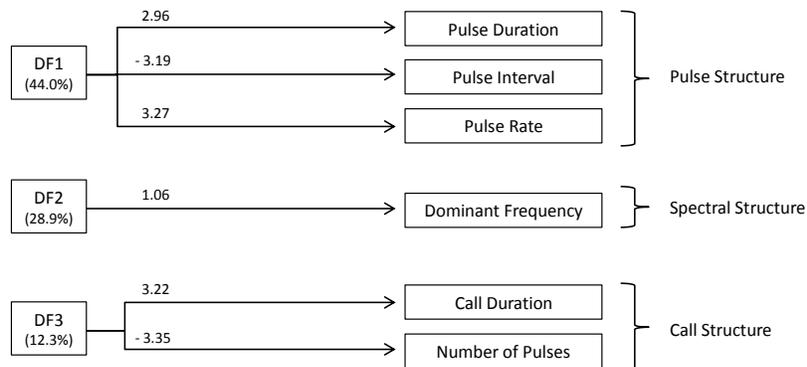
Figure 2.2: Combined results of principal components analysis (PCA) and discriminant function analysis (DFA). (a) shows DFA results using PCA scores as input variables and identifies the PCA factors that contribute the most towards each discriminant function's (DF) discrimination ability. Each DF label includes the percentage of variance in PCA factors explained. Each principle component (PC) label includes the percentage of variance in measured call properties explained. (b) shows DFA results using original scores (temperature corrected and transformed) as input variables. Numbers above DF arrows represent the associated standardized canonical discriminant function coefficients (from Table 4). Numbers above PC arrows represent the associated factor loadings (from Table 3).

Figure 2.2: con't

a. DFA using PCA scores as input



b. DFA using original values as input



SUPPLEMENTARY MATERIALS

A. Temperature Corrections

Because temperature is known to affect some call properties in anurans (Gerhardt & Huber 2002), we calculated Pearson's product moment correlations to assess the relationships between individual mean values of call properties and temperature. We calculated temperature correlations separately for our two datasets due to one set consisting of independent recordings (call variability) and the second set consisting of repeated recordings (estimates of repeatability). Associations were considered significant when the correlation between a call property and temperature was greater than 0.312, the critical r -value for the larger dataset (Supplementary Material, Table 2.SM1). For our call variability dataset ($N = 40$ males), we found pulse duration, pulse interval and call interval to be negatively correlated with temperature (Supplementary Material, Table 2.SM1, Fig. 2.SM1a, 2.SM1b & 2.SM1d). Similar to other anurans (reviewed in Gerhardt & Huber 2002), we found both pulse rate and call rate increased significantly with temperature (Supplementary Material, Table 2.SM1, Figs 2.SM1c & 2.SM1d). These positive relationships resulted because males produced shorter pulses with shorter pulse intervals and shorter calls (although not significant) with shorter call intervals as temperatures increased (Supplementary Material, Table 2.SM1, Figs 2.SM1a, 2.SM1b, 2.SM1d). In our smaller repeatability dataset, we found pulse duration to be positively correlated with temperature (Supplementary Material, Table 2.SM1, Fig. 2.SM1f). Following the procedure outlined by Platz and Forester (1988), we adjusted all properties

significantly correlated with temperature to 24° C, which was close to the mean air temperature of all recordings (24.8° C).

B. Discriminant Function Analysis (DFA) Using Biologically-Relevant Group Sizes

Previous studies examining individual distinctiveness using DFA have suggested that analyses involving large sample sizes potentially exaggerate the magnitude of the recognition problem faced by receivers by overestimating the number of calling males that a receptive female or territorial resident might realistically be required to distinguish at any one time (Bee et al. 2001; Bee & Gerhardt 2001a). Based on focal observations, individual *A. beebei* typically had between four and 10 territorial neighbors at any given time (Pettitt unpublished data). Females also maintain territories which are typically located adjacent to current mating partners (Bourne et al. 2001). Thus, analyses involving smaller sample sizes potentially better represent the realistic discrimination challenges faced by males (and possibly females) during territorial interactions and females during mate selection. Consequently, we modified our DFA to reflect the discrimination challenges faced by *A. beebei* receivers by re-measuring classification success using smaller, more biologically relevant sample sizes. To measure classification success we assigned every other call from a male to a ‘training’ dataset and a ‘test’ dataset. We generated discriminant functions using the training set and then used those functions to classify calls in the test set. We used a resampling procedure to determine the mean percentage of calls assigned to the correct male for 1000 groups each of four, six, eight or 10 individuals selected randomly with replacement from the larger

sample of 40 males. Estimates of classification success based on these biologically relevant group sizes found even higher percentages of correct assignments compared to estimates based on a group size of 40 males (79%). The mean and standard deviation (SD) values of classification success for group sizes of four, six, eight or 10 individuals were 98.7% (SD = 0.03), 96.6% (0.04), 95.0% (0.04), and 93.4% (0.04) respectively.

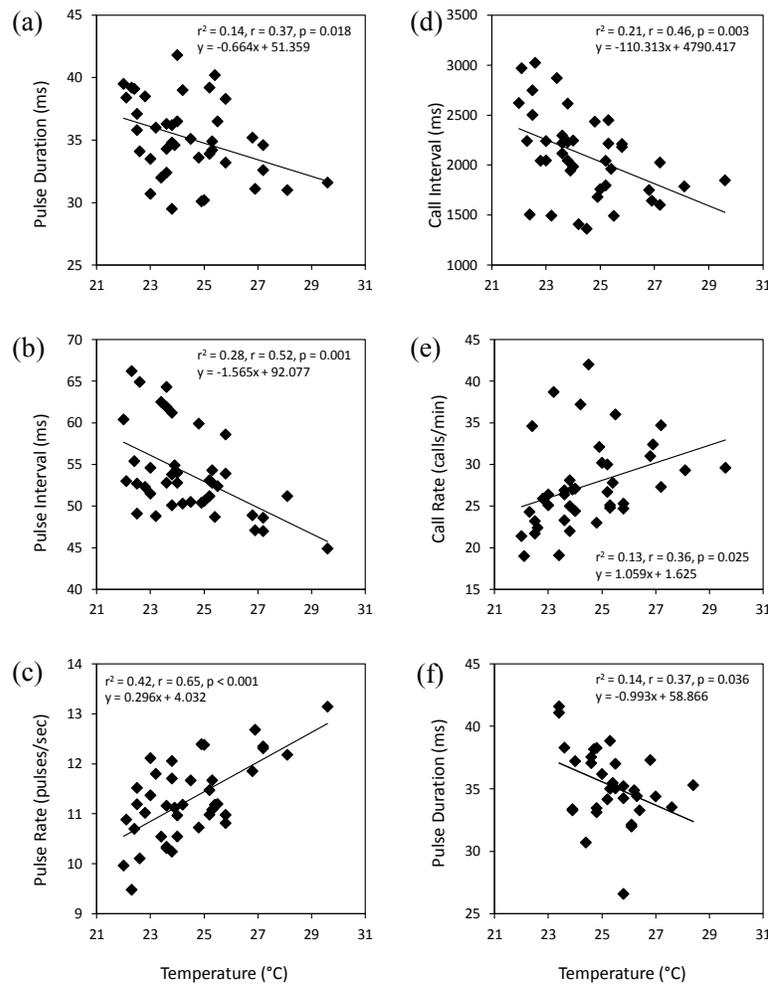
Supplementary Material – Table 2.SM1: Pearson’s correlation coefficients of call properties with air temperature in the golden rocket frog, *Anomaloglossus beebei*.

	Within- and Among- Variability Dataset (<i>N</i> = 40)	Repeatability Dataset (<i>N</i> = 16)
Pulse Duration (ms)	-0.374*	0.371*
Pulse Interval (ms)	-0.524**	0.028
Pulse Rate (pulses/sec)	0.649**	0.129
Call Duration (ms)	-0.267	0.229
Call Interval (s)	-0.456**	0.045
Call Rate (calls/min)	0.355*	0.093
Pulses Per Call	-0.041	0.260
Dominant Freq (kHz)	0.022	0.077

* *P* < 0.05, ** *P* < 0.01

Bold values indicate call properties corrected for temperature (see text for explanation)

Supplementary Material – Figure 2.SM1: Scatterplots of six temporal call properties versus air temperature. Shown here are the mean values from one dataset used to analyze call variability ($n = 40$ individuals) for (a) pulse duration (ms), (b) pulse interval (ms), (c) pulse rate (pulses/sec), (d) call interval (ms) and (e) call rate (calls/min). Also shown are mean values from a second dataset used to analyze repeatability ($n = 16$ individuals) for (f) pulse duration. The output from a linear regression of each call property on air temperature is shown along with the best-fit regression line.



**Chapter 3: Abiotic and biotic factors associated with egg and tadpole
deposition site selection and hatching success in a phytotelm-breeding
frog, *Anomaloglossus beebei***

SUMMARY

For many anurans, successful offspring development and survival depends on the environmental factors associated with offspring deposition sites. In some species, especially phytotelm-breeding species, parental frogs select separate sites for oviposition and tadpole rearing. We evaluate evidence for the hypothesis that parents of such species may benefit from using different criteria for selecting suitable habitat for eggs and tadpoles due to their different requirements for growth and survival. We investigated the importance of abiotic and biotic factors on oviposition versus tadpole rearing site selection as well as offspring survival in the phytotelm-breeding golden rocket frog, *Anomaloglossus beebei*. We monitored 132 bromeliads in which *A. beebei* live and breed almost exclusively. We measured the environmental factors associated with egg and tadpole deposition sites including: bromeliad size, leaf height, leaf angle (indicative of deposition site location within the bromeliad), water volume, dissolved oxygen, water temperature, presence/absence of predators (e.g., crabs, dragonfly larvae) and the presence/absence of nearby conspecifics. Compared to tadpole rearing sites, we found oviposition sites had higher dissolved oxygen concentrations, more crabs, smaller water volumes, lower temperatures and were found in leaves at lower angles within bromeliads. We found successful clutches were positively associated with dissolved oxygen and the presence of dragonfly larvae as well as nearby conspecifics and negatively associated with water temperature and the presence of predatory crabs. We conclude that the non-random pattern of microhabitat use for egg and tadpole deposition in *A. beebei* is

associated with specific environmental factors and that some of these same factors are directly related to *A. beebei* hatching success.

INTRODUCTION

Selecting suitable habitat for offspring development can influence an animal's reproductive success by directly impacting offspring growth and survival (Resetarits 1996; Lehtinen 2004). Selection should favor females and males that can assess local abiotic and biotic environmental factors that lead to successful offspring development. For most temperate anurans, offspring development occurs in medium-sized to large aquatic habitats including temporary and permanent ponds and streams (Duellman & Trueb 1994). For tropical species, offspring development often takes place in discrete microhabitats such as arboreal water pools, termed "phytotelmata," where resources and safety from predators can be limited (Wassersug et al. 1981; Lehtinen et al. 2004). In some species of phytotelm-breeding frogs, embryonic and larval development occurs in the same phytotelm. In others, especially those with more advanced forms of parental care, oviposition and tadpole rearing sites are distinct, with newly hatched tadpoles being transported between the two sites on the back of a parent. Because developing embryos and tadpoles have different requirements for growth and survival (Duellman & Trueb 1994), we hypothesize that parents of such species may benefit from using different criteria for selecting suitable habitat for the two different developmental stages.

Many abiotic factors are known to influence anuran growth and survival and thus may impact oviposition and tadpole rearing site selection including: water temperature (Howard 1980; Seale 1982; Waldman 1982; Caldwell 1986; Harkey & Semlitsch 1988), dissolved oxygen concentrations (Bradford & Seymour 1988), water volume or depth

(Crump 1991; Spieler & Linsenmair 1997), and vegetation structure (Howard 1978; Formanowicz & Bobka 1989; Babbitt & Tanner 1998). Likewise, biotic factors, such as presence of predators or nearby conspecifics, may also affect the probability of offspring survival (Howard 1980; Crump 1991; Resetarits & Wilbur 1989) and thus could impact a frog's habitat selection behavior. Individuals may avoid depositing eggs or tadpoles in sites that contain predators (Resetarits & Wilbur 1989; Rieger et al. 2004; Rudolf & Rödel 2005), while the presence of nearby conspecifics may increase offspring survival. For example, depositing offspring in areas with other developing conspecifics can reduce individual probability of predation due to increases in group size via the dilution effect (Rohwer 1978; Ridley & Rechten 1978).

A neotropical species that uses separate sites for oviposition and tadpole rearing, the golden rocket frog, *Anomaloglossus beebei*, exhibits biparental care and lives and breeds almost exclusively in giant tank bromeliads, *Brocchinia micrantha* (Bourne et al. 2001). Males are the primary care givers, providing egg and tadpole attendance, egg manipulation to reduce fungal infections, and tadpole transport between oviposition and tadpole rearing sites. While females occasionally lay unfertilized, trophic eggs in phytotelmata with developing tadpoles, nutritive egg provisioning is facultative in *A. beebei* and tadpoles also feed on fallen leaves and microorganisms (Bourne et al. 2001). During breeding, males attract females to their territories by producing an advertisement call. Approaching females are led to one or more phytotelmata (i.e., potential oviposition sites) by the male, but sometimes she will leave the male and inspect adjacent sites by herself (Pettitt personal observation). This behavior suggests that females exhibit

preferences for oviposition sites based on differences among phytotelmata, a phenomenon that has been observed in other phytotelm-breeding frogs (Jungfer & Weygoldt 1999; Alves-Silva & da Silva 2009). If the female accepts the site by remaining in the phytotelm, eggs will be deposited at or near the water line. Upon hatching, tadpoles are transported to another phytotelm by the male (Bourne et al. 2001).

In the present study, we examined evidence for our hypothesis that parents benefit from using different criteria for selecting egg and tadpole deposition sites by investigating the environmental factors associated with these two types of sites and the importance of these factors for offspring survival in the biparental golden rocket frog. Due to differences in life stage duration, motility, food requirements and respiratory activity between the anuran embryonic and larval stages, we expect to see differences in the factors associated with the two types of deposition sites selected by parental frogs and we expect these factors to impact offspring survival. Our goals were to: (i) provide a general description of the reproductive ecology of *A. beebei* and to use statistical models (ii) to compare the abiotic and biotic factors associated with oviposition versus tadpole rearing sites and (iii) to investigate the relationship between these factors and offspring survival (e.g., hatching success). Modeling factors that predict oviposition versus tadpole rearing sites evaluates evidence that adult *A. beebei* non-randomly select deposition sites for eggs and tadpoles and identifies factors that are important for distinguishing the two different types of sites. Modeling abiotic and biotic factors that predict hatching success identifies the environmental influences with the greatest impact on *A. beebei* offspring survival.

METHODS

A. Study site and organism

Oviposition and tadpole rearing sites were investigated during May-October 2009 and May-July 2010. Endemic to Guyana, South America, *A. beebei* is found only in Kaieteur National Park (Kok et al. 2006). We studied a free-ranging population in the park along the edge of a 455 m plateau near Kaieteur Falls (5°1'23"N, 59°28'52"W). Golden rocket frogs are commonly found in open glades near the falls where giant bromeliads are abundant and mist from the falls is continually present (Bourne et al., 2001). Breeding occurs year round, but peaks during the long rainy season between June and August (Bourne et al. 2001). Both males and females maintain territories that contain multiple phytotelmata. Although cannibalism by larger (stage 37-40; Gosner 1960) tadpoles has been observed, it is considered to be uncommon (Bourne et al. 2001).

B. Field Surveys and Behavioral Observations

We examined a total of 132 bromeliads and identified 211 phytotelmata with egg clutches (i.e., oviposition sites) and 105 phytotelmata with larvae (i.e., tadpole rearing sites). We used count data from all phytotelmata ($N = 316$) to compute the frequencies of: (i) single and multiple egg clutches in each bromeliad ($N = 132$) and in each phytotelm containing eggs ($N = 211$) and (ii) single and multiple tadpoles in each bromeliad ($N = 132$) and in each phytotelm containing tadpoles ($N = 105$). Due to time constraints,

unknown dates of oviposition and to avoid pseudoreplication, we collected data on offspring survival as well as abiotic and biotic factors associated with deposition sites using a subset ($N = 144$) of these phytotelmata in 132 bromeliads. For example, when there was more than one egg clutch or more than one tadpole per bromeliad, we limited our analyses of abiotic and biotic factors associated with a single clutch or a single tadpole to avoid pseudoreplication. Of this subset of 144 phytotelmata, we took daily surveys of 108 oviposition sites and 36 tadpole rearing sites. We identified the oviposition sites by first marking the location of active courtships and returning the following day to locate newly laid eggs. We identified the tadpole rearing sites by searching bromeliads haphazardly. During each survey we took counts of the number of eggs, tadpoles, and adults (if present) by visually examining all leaves and leaf axils. We also captured adults when possible and clipped a unique number of toes for identification. We surveyed only plants that included the territory of one male which we determined based on repeatedly seeing or capturing a single territorial male within a plant.

To describe the reproductive ecology of *A. beebei*, we collected data on clutch size ($N = 108$ clutches), fate of egg clutches ($N = 54$ clutches), egg size ($N = 14$ one-day old eggs from 5 different clutches), and time to hatching ($N = 40$ eggs). We used data from 96 oviposition sites and 36 tadpole rearing sites to compare the two deposition sites. Twelve oviposition sites from the original 108 were excluded from this analysis to maintain independence because these sites were in bromeliads in which we collected data from both an oviposition and a tadpole rearing site. We used data from 54 oviposition sites to identify the abiotic and biotic factors with the greatest impact on offspring

survival. We excluded half ($N = 54$) of the original 108 oviposition sites because the fathers of these clutches were removed as part of a larger male removal study. Because very few of the surveyed phytotelmata had more than one egg clutch (2 out of 108) and none had more than one tadpole (0 out of 36) we were unable to examine the importance of conspecific competition on deposition site selection and offspring survival. However, we did examine the importance of the presence of nearby developing conspecifics (i.e., eggs or tadpoles in phytotelmata within the same bromeliad).

We recorded the following abiotic factors associated with oviposition and tadpole rearing sites: (i) number of living, green leaves as a measure of bromeliad size (SIZE), (ii) leaf height (LH; ± 1 cm) measured from the ground to the phytotelm water line, (iii) leaf angle (LA; visually estimated to the nearest 0° , 30° , 45° , 60° , or 90° from horizontal), (iv) water volume (WV; ± 5 ml), (v) dissolved oxygen concentration (DO; ± 0.01 parts per million (ppm)), and (vi) water temperature (WT; $\pm 0.1^\circ\text{C}$). We measured leaf angle for two reasons. First, preliminary studies with *A. beebei* suggested both eggs and egg predators were more commonly found in phytotelmata at lower leaf angles and tadpoles were more commonly found in phytotelmata at higher leaf angles (Pettitt unpublished data). Terrestrial bromeliad leaves grow out of the center of the plant as stiff, green, upward-pointing leaves. As the leaves age, they are pushed to the outside by newer leaves and slowly bend downwards until they reach a horizontal angle with the forest floor at their axes. Such older leaves are typically brown and dry and are often surrounded by overhanging green leaves (Fig. 3.1). We considered “lower leaf angles” to be $0 - 45^\circ$ from horizontal and “higher leaf angles” to be $60 - 90^\circ$ from horizontal. Second, leaf

axils at lower angles are known to form more discrete water bodies while inner leaf axils at higher angles often combine to form a common pool (Frank 1983), suggesting that water availability in leaf axils at higher angles may be more consistent than in leaf axils at lower angles. Dissolved oxygen and water temperature were measured between 1600-1800 h using an Extech DO600 dissolved oxygen meter (Davis Instruments, Vernon Hills, IL). Water volume was measured by drawing the water out using a 40 mL plastic turkey baster with an enlarged opening to allow suction catching of tadpoles without injury (Bourne et al. 2001). We collected the water in a large ziplock bag until the phytotelm was empty and immediately returned it and all debris and organisms collected. We also recorded the following biotic variables for all phytotelmata containing egg clutches or tadpoles: (i) presence/absence of predators and (ii) presence/absence of previously laid egg clutches or developing tadpoles in other phytotelmata within the same bromeliad. Predators of *A. beebei* include grapsid crabs, colubrid snakes (*Imantodes* sp.), Drosophilidae fly larvae and dragonfly larvae (Bourne et al. 2001; Pettitt personal observation). We included in our analyses the two most common egg and tadpole predators: grapsid crabs (CRAB) and dragonfly larva (DFLY) as well as the presence of conspecific eggs or tadpoles in other phytotelmata within the same plant (CON).

We monitored the fate of egg clutches as well as time to hatching for individual eggs. We categorized the fate of clutches as: (i) predation, (ii) fungal infection, (iii), desiccation, (iv) failure to develop, (v) hatched or (vi) unknown. Eggs were considered depredated if embryos were missing less than 11 days following fertilization. After 11 days, the earliest age of known hatching, the fate of missing embryos was categorized as

unknown because we did not know if the embryos hatched or were depredated. We considered that eggs had died due to a fungal infection when we saw hyphae covering the eggs, desiccation when the eggs had shriveled and become dehydrated, and failed to develop when embryonic growth was suspended for more than one week. Although most eggs whose embryos failed to develop eventually showed signs of fungal infection, we categorized their fate as failing to develop. We identified eggs that had successfully hatched when we directly observed the hatching or when we saw an egg capsule with a missing embryo the same day as we saw a new small black tadpole in the same phytotelm. When the eggs within a clutch experienced more than one fate, we recorded the most common fate for the clutch as whole. We measured egg survival in terms of clutch success. We considered a clutch successful if one or more eggs hatched.

C. Statistical Analyses

All statistical analyses were conducted in SPSS v12.0 (SPSS, Inc., Chicago, IL, U.S.A.). We conducted univariate analyses to describe the general reproductive ecology of *A. beebei*. We conducted and report findings from both model selection and univariate analyses that identify environmental influences on *A. beebei* deposition site selection and offspring survival. We recognize our model selection based approach as the most effective way to distinguish among candidate models consisting of different combinations of environmental influences. We present univariate results for readers who prefer to evaluate the individual effects of environmental influences via their statistical significance. We used t tests for continuous abiotic factors and Pearson chi-square tests

for categorical biotic factors. If abiotic factors could not be transformed to take on a normal distribution, a Mann-Whitney U test was used.

We used multiple logistic regression models to analyze the importance of abiotic and biotic factors for: (i) the selection of oviposition versus tadpole rearing sites and (ii) hatching success. For both analyses we used an information theoretic approach to determine the most parsimonious explanatory models (Burnham & Anderson 2002; Mazerolle 2006). Using nine predictor variables (i.e., six abiotic factors and three biotic factors; Table 3.1), we created several potential explanatory models. We selected the best approximating models with likelihood-based methods using Akaike Information Criterion after correction for small sample size (AICc; <40 observations/variable in the largest model). The goal of AICc is not to determine the significance of any particular model, but to compare a suite of *a priori* based candidate models to determine which combinations of predictor variables most parsimoniously predict the response variable. Best models can then be used for inference and further exploration (Burnham & Anderson 2002). Models with large number of variables are penalized in AICc because a model's predictive power decreases with too many predictor variables (Burnham & Anderson 2002).

We did not test all combinations of all nine variables using AICc because it is recommended to minimize the number of candidate models by using previous knowledge of the system (Burnham & Anderson 2002). For example, when creating models to predict hatching success, we included variables that are known to influence anuran egg development and survival (e.g., dissolved oxygen concentration, water temperature,

presence of predators). In addition to these *a priori* based models ($N = 9$ models predicting deposition site selection; $N = 6$ models predicting hatching success), we also tested a global model (i.e., including all nine variables) as well as a model with all abiotic variables and a model with all biotic variables. Although some of the nine predictor variables were significantly correlated with one another, the correlation coefficients were never high enough ($|r| < 0.6$) to exclude any variables from the analyses (Tabachnick & Fidell 1996). For each model, all selected variables were simultaneously entered (i.e., stepwise procedures were not used) and we tested for over dispersion of the data using the Hosmer-Lemeshow Goodness-of-fit procedure (Hosmer & Lemeshow 2000). The test was non-significant for all models ($ps > 0.12$). We ranked models by comparing ΔAIC_c values which were calculated as $\Delta AIC_c = AIC_{c_i} - AIC_{c_{min}}$ where $AIC_{c_{min}}$ is the model with the lowest AICc value and i indicates other competing models (Burnham & Anderson 2002). The best model has the lowest AICc value and therefore has a ΔAIC_c of zero. We used Akaike weights (w_i) to assess the weight of evidence in favor of each model and to evaluate model selection uncertainty. Models with similar weights are equally plausible for a given data set (Burnham & Anderson 2002). To quantify the evidence for the importance of each factor, we summed the Akaike weights across all of the models in each of the two analyses where a given factor occurred (Burnham & Anderson 2002). Nagelkerke R^2 values were used to assess the predictive performance of the logistic regression models.

RESULTS

A. Reproductive Ecology

We monitored 44 bromeliads during 2009 and 88 during 2010. Of these 132 *bromeliads*, which each included the territory of only one male, 55% contained only 1 egg clutch ($N = 73$), 27% contained 2 clutches ($N = 36$), 15% contained 3 clutches ($N = 20$), and 3% contained 4, 7 or 9 clutches simultaneously ($N = 3$). In terms of tadpoles, 73% of the 132 bromeliads contained no tadpoles ($N = 96$), 9% contained 1 tadpole ($N = 12$), 4% contained 2 tadpoles ($N = 5$), 4% contained 3 tadpoles ($N = 5$) and 10% contained 4 - 12 tadpoles ($N = 14$). Of all *phytotelmata* that contained egg clutches ($N = 211$) in all bromeliads, 94% had only one clutch ($N = 199$), 5% had two clutches ($N = 10$) and 1% had three clutches simultaneously ($N = 2$). Of all *phytotelmata* that contained tadpoles ($N = 105$) in all bromeliads, 84% had only one tadpole ($N = 88$), 10% had two tadpoles ($N = 11$) and 6% had 3 - 7 tadpoles present simultaneously ($N = 6$). Both egg clutches and tadpoles were found in all sizes of bromeliad (i.e., number of green leaves ranged from 4 – 30 per plant) and in leaves that ranged from 8-147 cm above the ground. While 88% of egg clutches were found in *phytotelmata* at lower leaf angles (0° - 45°), 75% of tadpoles were found in *phytotelmata* at higher leaf angles (60° - 90°). Most crabs (81%; $N = 30$ of 37) were found in lower leaf angles (0° - 45°), while dragonfly larva were found most commonly in leaf angles of 30° - 60° (89%; $N = 24$ of 27). Mean and standard deviation (SD) clutch size was 4.8 ± 1.1 eggs ($N = 108$; range = 2 – 8). Mean and SD diameter of one day old eggs was 9.5 ± 1.9 mm ($N = 14$ eggs from 5 different clutches; range = 6.6 – 12.7 mm). Embryos hatched within 14.7 ± 2.3 days ($N = 40$; range = 11 –

18) after deposition. While egg clutches in phytotelmata with crabs ($N = 5$) hatched earlier than those without crabs ($N = 35$), the difference was not significant (mean = 13.8 and 14.8 days, respectively; $t = 0.92$, $df = 38$, $P = 0.36$).

Fifteen of 54 egg clutches (28%) investigated yielded one or more tadpoles and were considered successful. The fates of the remaining clutches were related to depredation (44%), fungal infection (22%), desiccation (2%), failure to develop (2%) or unknown causes (2%). Common organisms found in phytotelmata containing *A. beebei* eggs and tadpoles included dragonfly larvae, mosquito larvae, grapsid crabs, and *Tepuihyla* spp. Ants, mosquitos and small flies were found on the surface of the leaves above the water line. Grapsid crabs were found in 26% (37/144) of phytotelmata that contained either eggs or tadpoles, while dragonfly larvae were found in 19% (27/144) of these phytotelmata. Both crabs and dragonfly larvae were found more often in larger bromeliads ($t = -2.42$, $df = 142$, $P = 0.02$ and $t = -3.43$, $df = 142$, $P = 0.001$, respectively). Egg clutches in phytotelmata with crabs, but not dragonfly larvae, were more likely to have one or more embryos depredated (Pearson chi-square tests, $\chi^2 = 9.50$, $df = 1$, $P = 0.002$ and $\chi^2 = 0.64$, $df = 1$, $P = 0.42$, respectively). There was a non-significant trend for dragonfly larvae to be found more often in phytotelmata with higher water temperatures ($t = -1.73$, $df = 125$, $P = 0.09$).

B. Importance of Abiotic and Biotic Factors: Oviposition versus Tadpole Rearing Site Selection

Based on the weighted AICc (w_i) values for models predicting selection of oviposition versus tadpole rearing sites, models 1 and 2 accounted for 94% of the Akaike weight and were approximately 13 and 10 times better, respectively, than the next best model (i.e., model 3; Table 3.2). The top two models explained 62% and 63% of variance in the data (Nagelkerke's R^2 ; Table 3.2), respectively, and suggested that *A. beebei* adults do not deposit eggs and tadpoles randomly. The remaining alternative models had much lower support than the best two models. Model 1 contained four factors which also had the highest summed Akaike weights: leaf angle ($\Sigma w_i = 0.9999$), dissolved oxygen ($\Sigma w_i = 0.9998$), water volume ($\Sigma w_i = 0.9782$) and presence of crabs ($\Sigma w_i = 0.9968$; Tables 3.3 and 3.4). Model 2 differed from model 1 by the addition of a single factor, water temperature, which had the next highest summed Akaike weight ($\Sigma w_i = 0.4783$). Logistic regression coefficients suggested that oviposition sites had higher dissolved oxygen concentrations, more crabs, smaller water volumes, lower temperatures and were found in leaves at lower angles than tadpole rearing sites (Table 3.3).

We report the mean and SD of abiotic factors and percentages for biotic factors at *A. beebei* oviposition and tadpole rearing sites in Table 3.2. Univariate tests showed that compared with tadpole rearing sites, oviposition sites had smaller water volumes, higher concentrations of dissolved oxygen, lower leaf angles, more crabs and fewer nearby conspecifics (Table 3.5).

C. Importance of Abiotic and Biotic Factors: Offspring Survival

According to our AICc analyses, the top two models describing the importance of microhabitat factors on hatching success accounted for 77% of the Akaike weight and were both approximately three times better than the next best model (i.e., model 3; Table 3.6). These top two models explained 29% and 36% of variance in the data (Nagelkerke's R^2 ; Table 3.6), respectively. Our best model contained four factors which also had the highest summed Akaike weights: dissolved oxygen ($\Sigma w_i = 0.9818$), water temperature ($\Sigma w_i = 0.9818$), and the presence of dragonfly larvae ($\Sigma w_i = 0.9048$) and nearby conspecifics ($\Sigma w_i = 0.8990$; Tables 3.5 and 3.7). Model 2 differed from model 1 by the addition of a single factor, presence of crabs. This additional factor had a much lower summed Akaike weight ($\Sigma w_i = 0.3854$). Logistic regression coefficients suggested that successful clutches were positively associated with dissolved oxygen and presence of dragonfly larvae and nearby conspecifics and negatively associated with water temperature and the presence of crabs (Table 3.4).

We report the mean and SD of abiotic factors and percentages for biotic factors associated with successful and unsuccessful *A. beebei* egg clutches in Table 3.2. Univariate results showed that sites with successful and unsuccessful clutches did not differ from each other significantly, but there was a trend for successful clutches to be found in phytotelmata with dragonfly larvae ($p = 0.051$) and previously laid conspecific egg clutches nearby ($p = 0.050$; Table 3.7).

DISCUSSION

This study reveals two important aspects about the use of breeding habitat by *A. beebei*. First, our results indicate that the non-random pattern of microhabitat use for egg and tadpole deposition in *A. beebei* is associated with specific abiotic and biotic factors that are known to influence anuran offspring development and survival. Second, we show that some of these same factors are directly related to *A. beebei* hatching success. These findings suggest that adult *A. beebei* use some microhabitat factors as cues to select deposition sites and that this selection behavior may benefit parents by increasing offspring survival.

A. Importance of Abiotic and Biotic Factors: Oviposition versus Tadpole Rearing Site Selection

Our top two models indicated that leaf angle (LA), water volume (WV), dissolved oxygen (DO), water temperature (WT), and presence of predatory crabs (CRABS) were most important in explaining the difference between sites selected for egg and tadpole deposition by adult *A. beebei* (Table 3.2). Oviposition sites were associated more with phytotelmata at lower leaf angles (i.e., around the base of the plant at 0-45° from horizontal) compared to tadpole rearing sites (60 – 90° from horizontal). Because leaf axils at lower angles form more discrete water bodies compared to inner leaf axils at higher angles that are often connected hydrologically (Frank 1983), we would expect phytotelmata at lower angles to be more likely to dry out during periods with little rain. Consequently, *A. beebei* egg clutches may face higher desiccation risks than tadpoles due to the leaf angle associated with their respective deposition sites. Oviposition sites were

also associated with smaller volumes of water compared to tadpole rearing sites. Although both eggs and tadpoles are vulnerable to desiccation (Duellman & Trueb 1994), the length of time each developmental stage is vulnerable is different. In *A. beebei*, eggs hatch between 11-18 days after deposition and are often immediately transported to different phytotelmata. Tadpoles, on the other hand, can require more than six months before metamorphosis (Pettitt unpublished data) and rely on water stored in phytotelmata for the duration of their development (Bourne et al. 2001). In addition, phytotelmata containing small amounts of water are typically associated with low nutrient levels (Wassersug et al. 1981; Crump 1992) and thus, larger volumes should represent greater nutrient resources and enhanced tadpole growth. The nutrient needs of anuran embryos are provided for by the egg yolk (Duellman & Trueb 1994). Consequently, a greater and more stable volume of water could be especially important for tadpole development and survival. Similar results were found in *Ranitomeya variabilis*, another bromeliad-breeding species, where males prefer to deposit tadpoles in larger pools while smaller pools are used for egg deposition (Brown et al. 2008).

Our findings suggested that dissolved oxygen concentrations and water temperature were also important differences between oviposition and tadpole rearing sites. Higher dissolved oxygen concentrations and lower water temperatures were more closely associated with oviposition than tadpole rearing sites. Although these two factors are known to be inversely proportional, with cooler water containing more dissolved oxygen (Dejours 1981), the phytotelmata included in this study did not show a significant correlation between water temperature and dissolved oxygen (Pearson's $r = 0.06$, $P =$

0.50). The lack of a relationship between these two factors could be due to the influence of other environmental factors including rainfall (Benson & Krause 1984) and amount of decomposing material in the phytotelm (Kam et al. 1996). The presence of dissolved oxygen is important to the development of both anuran embryos (Seymour & Bradford 1987) and tadpoles (Wassersug & Feder 1983). However, unlike embryos in their egg capsules, tadpoles are mobile and most, including *A. beebei* tadpoles, can consume oxygen by gulping air (Duellman & Trueb 1994; Bourne et al. 2001). Thus, deposition sites with high dissolved oxygen concentrations may be more critical for successful embryonic development than for tadpole development due to alternative strategies available to tadpoles for obtaining sufficient amounts of oxygen. Finding tadpoles in phytotelmata with lower concentrations of dissolved oxygen may also be related to other factors that we did not measure. For example, leaf litter, which decreases dissolved oxygen concentrations as a result of microbial decomposition (Hargrave 1972), is a potential source of food and shelter for tadpoles, but not for embryos.

Lastly, we found that the presence of predatory crabs played an important role in modeling oviposition versus tadpole rearing site selection. While grapsid crabs will attack and consume both *A. beebei* eggs and tadpoles (Bourne et al. 2001), crabs were more often associated with oviposition sites. There are multiple explanations for this relationship. First, similar to egg clutches, crabs are more often found in leaf axils with lower angles (e.g., 0 – 45° from horizontal). We often observed crabs move between leaf axils at lower angles while their attempts to move into leaf axils at higher angles (60 - 90°) almost always failed (Pettitt personal observation). Second, the presence of crabs

could be related to other abiotic factors that we did not measure during this study. One bromeliad-breeding grapsid crab (*Metopaulias depressus*) selects breeding sites by accessing not only the physical characteristics of the leaf axils, but also the chemical conditions of the phytotelmata (Diesel & Schuh 1993). Finding crabs to be more often associated with oviposition sites compared to tadpole rearing sites may be a result of these sites having conditions that are good for the successful development of both anuran embryos as well as the offspring of arthropods.

B. Importance of Abiotic and Biotic Factors: Offspring Survival

Our top two models indicated that dissolved oxygen (DO), water temperature (WT), presence of both crabs (CRABS) and dragonfly larvae (DFLY) and presence of nearby conspecifics (CON) were most important in explaining the differences between sites with successful and unsuccessful *A. beebei* clutches (Table 3.6). We found unsuccessful clutches to be associated with lower dissolved oxygen concentrations (Table 3.4). Oxygenation of anuran eggs is critical to embryonic development and low concentrations of dissolved oxygen can constrain normal growth rates (Salthe & Mecham 1974). A decrease in rates of development increases the amount of time that developing embryos are vulnerable to predation and desiccation (Voss 1993). We also found unsuccessful clutches to be associated with higher water temperatures. The influence of temperature on anuran hatching success is complex. While colder water typically contains more dissolved oxygen (Dejours 1981), which is important for normal embryonic development, it may also decrease metabolic rates slowing down rates of developmental and increasing

incubation time (Bachmann 1969). Ultimately, an embryo's rate of development at a given temperature is influenced by the temperature range to which it is adapted (Bachmann 1969). Optimal temperatures for the successful development of *A. beebei* embryos may be determined by a set of complex interactions between water temperature, dissolved oxygen and the various metabolic processes associated with embryonic growth.

Successful *A. beebei* clutches were negatively associated with the presence of crabs and positively associated with the presence of dragonfly larvae. These results are consistent with our univariate comparisons showing that clutches deposited in phytotelmata with crabs, but not dragonfly larvae, were more likely to have one or more eggs depredated. Many studies have found a negative effect of various predators on anuran egg survival, including but not limited to birds (Roberts 1994), snakes (Warkentin 1995), fish (Grubb 1972; Resetarits & Wilbur 1989), insects (Henrikson 1990; Warkentin 2000; Lips 2001), and conspecific and heterospecific anurans (Crump 1983; Drewes & Altig 1996). Although there are very few accounts of crabs as anuran predators for either eggs (Hayes 1983) or tadpoles and adults (Gray & Christy 2000), grapsid crabs are known to consume *A. beebei* eggs, tadpoles and adults (Bourne et al. 2001).

Consequently, we were not surprised to find that the presence of crabs had detrimental effects on hatching success in *A. beebei*. Dragonfly larvae, on the other hand, are important predators of *larval* anurans that inhabit temporary habitats (Smith 1983; Van Buskirk 1988). Because they are visual predators and attack moving prey (Pritchard 1965), dragonfly larvae do not often play an important role in egg predation (Magnusson and Hero 1991; Resetarits 1998). In this study, the presence of dragonfly larvae was

positively associated with hatching success. This relationship could be related to shared offspring development requirements; optimal dragonfly larva habitat may be related to environmental factors that also enhance anuran embryonic development (e.g., higher dissolved oxygen concentrations). Further studies are needed to more fully elucidate the relationships among dragonfly larvae, *A. beebei* hatching success and the environmental factors associated with phytotelmata.

Because adult *A. beebei* rarely deposit fertilized eggs in phytotelmata containing previously laid eggs or developing tadpoles, we were not able to examine the importance of conspecifics on hatching success in terms direct competition and potential risks of cannibalism. We were able to include in our models the presence of nearby conspecifics (i.e., eggs and tadpoles in phytotelmata within the same bromeliad, but not in the same phytotelm) which we found to be positively related to *A. beebei* hatching success. We suggest four potential, non-mutually exclusive explanations for this association. First, nearby conspecifics may increase offspring survival via the dilution effect (reduced individual probability of predation due to increased group size; Rohwer 1978). However, this explanation assumes that egg predators have equal access to all potential prey items, which may not be the case for all *A. beebei* egg predators. Grapsid crabs are capable of moving between phytotelmata, but typically only those in leaves at lower angles (Pettitt personal observation). Second, this relationship may result from environmental factors that are shared by all phytotelmata within a bromeliad. For example, a bromeliad located very close to Kaieteur Falls may offer favorable breeding conditions such as a consistent water supply within all its phytotelmata and consequently, successful offspring survival

in one area of the plant would be positively correlated with successful offspring survival in other areas of the same plant. Third, if all developing conspecifics found within a bromeliad are sired by the same male, successful offspring development may be due to the superior genetic quality of the territorial male (Howard 1991). In this study, we included all nearby conspecifics found within the same plant, however, there is a possibility that some offspring may have been sired by different males. Further studies addressing the existence of multiple paternity in *A. beebei* are needed to assess the potential for this explanation. Fourth, the positive association between hatching success and the presence of nearby conspecifics may be due to the superior parental care quality provided by the territorial male (Ridley 1978; Sargent 1988).

C. Conclusions

When comparing our modeling results identifying the abiotic and biotic factors most important in explaining hatching success and oviposition versus tadpole rearing site selection, we found three factors were common in our top models: dissolved oxygen, water temperature and the presence of crabs (Tables 3.3 and 3.5). Higher dissolved oxygen concentrations and lower water temperatures were more closely associated with oviposition sites compared to tadpole rearing sites and these same factors were associated with successful hatching. Additional studies of habitat selection in other anurans that use separate deposition sites for eggs and tadpoles are needed to evaluate the potential benefits received by parents that use different criteria when selecting deposition sites for offspring at different developmental stages.

Table 3.1: Codes and descriptions of abiotic and biotic factors quantified within *Anomaloglossus beebei* oviposition and tadpole rearing sites.

Factor	Code	Description
Water temperature	WT	Phytotelm water temperature in °C
Water volume	WV	Phytotelm water volume in ml
Dissolved oxygen	DO	Phytotelm dissolved oxygen concentration in parts per million
Leaf angle	LA	Angle of leaf measured as ° from horizontal
Phytotelm height	PH	Height at water line of phytotelm from ground in cm
Bromeliad size	SIZE	Plant size based on number of green leaves
Crabs	CRAB	Presence of crabs in phytotelm
Dragonfly larva	DFLY	Presence of dragonfly larvae in phytotelm
Conspecifics	CON	Presence of previously laid eggs or tadpoles in same plant

Table 3.2: Log likelihood and Akaike’s information criterion corrected for small sample sizes (AIC_c) for 12 *a priori* habitat models to predict microhabitat factors (described in Table 1) associated with oviposition sites ($N = 96$) versus tadpole rearing sites ($N = 36$).

#	Model	K	AIC_c^a	ΔAIC_c	Likelihood d	w_i^b	R^2_N
1	LA, CRAB, DO, WV	6	83.85	0.00	1.00	0.522	0.62
2	LA, CRAB, DO, WV, WT	7	84.31	0.46	0.79	0.415	0.63
3	WT, DO, LA, WV, PH, SIZE, CRAB, DFLY, CON	11	89.04	5.19	0.07	0.039	0.67
4	LA, CRAB, WT, DO	6	91.26	7.41	0.02	0.013	0.57
5	LA, CRAB, WT, DO, DFLY	7	92.02	8.17	0.02	0.003	0.58
6	WT, DO, WV, SIZE, PH, LA	8	94.07	10.22	0.01	0.000	0.58
7	LA, CRAB, DFLY, CON	6	100.11	16.26	0.00	0.000	0.58
8	LA, CRAB, DFLY	5	106.47	22.62	0.00	0.000	0.52
9	WT, DO, WV, SIZE, PH	7	122.88	39.03	0.00	0.000	0.31
10	WT, DO	4	127.23	43.38	0.00	0.000	0.20
11	CRAB, DFLY, CON	5	141.45	57.60	0.00	0.000	0.24
12	WV, SIZE, PH	5	158.61	74.76	0.00	0.000	0.07

K equals the number of parameters plus an intercept and error term, ΔAIC_c is the difference in AIC_c value between the model of interest and the model with the lowest AIC_c value, and w_i is the Akaike weight for model i . R^2_N is the Nagelkerke R^2 value used to assess a model’s predictive performance.

Table 3.3: Summed Akaike weights (Σw_i) for all 9 microhabitat factors and logistic regression model coefficients (β_i) and standard errors (SE), and for 5 explanatory factors of the best two models for microhabitat factors associated with oviposition versus tadpole rearing sites.

Factor	Σw_i	β_i	Model 1	Model 2	
			SE	β_i	SE
WT	0.4783			-0.235	0.176
WV	0.9782	-0.004	0.001	-0.004	0.001
DO	0.9998	0.837	0.272	0.881	0.279
LA	0.9999	-0.076	0.018	-0.078	0.019
PH	0.0421				
SIZE	0.0421				
CRAB	0.9968	2.364	1.006	2.444	1.030
DFLY	0.0479				
CON	0.0389				
CONSTANT		2.893	1.200	9.020	4.811

Table 3.4: Summed Akaike weights (Σw_i) for all 9 microhabitat factors and logistic regression model coefficients (β_i) and standard errors (SE), and for 6 explanatory factors of the best two models for microhabitat factors associated with hatching success.

Factor	Σw_i	β_i	Model 1		Model 2	
			SE	β_i	SE	
WT	0.9818	-0.181	0.198	-0.232	0.212	
WV	0.1391					
DO	0.9818	0.292	0.333	0.435	0.360	
LA	0.0048					
PH	0.0048					
SIZE	0.0048					
CRAB	0.3854			-1.402	0.908	
DFLY	0.9048	1.686	0.841	1.639	0.868	
CON	0.8990	1.775	0.851	1.839	0.897	
CONSTANT		1.661	4.993	2.928	5.315	

Table 3.5: Mean \pm standard deviation of abiotic factors and percentages for biotic factors at *A. beebei* oviposition and tadpole rearing sites. Microhabitat factors are described in Table 1.

Factor	<i>N</i>	Oviposition Site	<i>N</i>	Tadpole Rearing Site	Test Statistic	<i>df</i>	<i>P</i>
WT	88	26.2 \pm 1.9	31	26.5 \pm 1.3	0.87 ^a	117	0.39
WV	96	237 \pm 209	36	346 \pm 231	1170.0 ^b	130	0.004
DO	88	3.7 \pm 1.1	31	2.6 \pm 1.3	-4.28 ^a	117	<0.001
LA	96	31.7 \pm 21.3	36	64.2 \pm 19.5	433.0 ^b	130	<0.001
PH	96	69.0 \pm 34.1	36	75.4 \pm 31.0	0.99 ^a	130	0.32
SIZE	96	12.9 \pm 5.6	36	14.9 \pm 5.6	1345.5 ^b	130	0.050
CRAB	96	33%	36	8%	8.40 ^c	1	0.004
DFLY	96	17%	36	22%	0.54 ^c	1	0.46
CON	96	25%	36	61%	15.04 ^c	1	<0.001

^a T-test statistic

^b Mann-Whitney U test statistic

^c Pearson chi-square test statistic

Table 3.6: Log likelihood and Akaike’s information criterion corrected for small sample sizes (AIC_c) for nine *a priori* habitat models to predict microhabitat factors (described in Table 1) associated with successful clutches ($N = 15$) and unsuccessful clutches ($N = 39$).

#	Model	K	AIC_c^a	ΔAIC	Likelihood	w_i^b	R^2_N
1	DO, WT, CON, DFLY	6	58.07	0.00	1.00	0.405	0.29
2	DO, WT, CON, DFLY, CRAB	7	58.29	0.21	0.90	0.365	0.36
3	DO, WT, CON, DFLY, WV	7	60.61	2.53	0.28	0.114	0.30
4	DO, WT	4	61.53	3.45	0.18	0.072	0.03
5	DO, WT, WV	5	64.08	6.00	0.05	0.020	0.03
6	CON, DFLY, CRAB	5	65.04	6.96	0.03	0.013	0.24
7	DFLY, CRAB	4	66.58	8.50	0.01	0.006	0.15
8	DO, WT, WV, SIZE, PH, LA	8	68.08	10.00	0.01	0.003	0.18
9	DO, WT, WV, SIZE, PH, LA, CON, DFLY, CRAB	11	68.66	10.58	0.01	0.002	0.43

K equals the number of parameters plus an intercept and error term, ΔAIC_c is the difference in AIC_c value between the model of interest and the model with the lowest AIC_c value, and w_i is the Akaike weight for model i . R^2_N is the Nagelkerke R^2 value used to assess a model’s predictive performance.

Table 3.7: Mean \pm standard deviation of abiotic factors and percentages for biotic factors at *A. beebei* oviposition sites with successful and unsuccessful clutches. Microhabitat factors are described in Table 1.

Factor	<i>N</i>	Successful Clutches	<i>N</i>	Unsuccessful Clutches	Test Statistic	<i>df</i>	<i>P</i>
WT	14	26.7 \pm 2.2	28	26.8 \pm 1.9	0.22 ^a	40	0.83
WV	15	233 \pm 178	39	215 \pm 205	319.0 ^b	52	0.61
DO	14	3.6 \pm 1.3	28	3.2 \pm 1.2	-0.90 ^a	40	0.37
LA	15	28.0 \pm 19.5	39	27.7 \pm 16.7	309.5	52	0.72
PH	15	70.1 \pm 41.8	39	65.1 \pm 32.3	-0.47 ^a	52	0.64
SIZE	15	15.3 \pm 7.1	39	12.9 \pm 6.0	357.5	52	0.21
CRAB	15	20%	39	38%	1.66	1	0.20
DFLY	15	40%	39	15%	3.80	1	0.051
CON	15	73%	39	44%	3.84	1	0.050

^a T-test

^b Mann-Whitney U test

^c Pearson chi-square test

Figure 3.1: Giant terrestrial bromeliad, *Brocchinia micrantha*.



**Chapter 4: Paternal Effort in Relation to Acoustically Mediated Mate
Choice in a Neotropical Frog**

SUMMARY

Male secondary sexual traits may be indicators of direct or indirect fitness benefits to females that mate with those males. Direct benefits, such as parental care, can be especially important to females in species with biparental care. Four main hypotheses have been proposed to explain the relationship between male advertisement traits and paternal care quality. The good parent hypothesis predicts that males with more attractive traits will provide more care, while the differential allocation hypothesis predicts that they will provide less care. The trade-off hypothesis also predicts less care, but only when mating opportunities are numerous. The essential male care hypothesis predicts males will reliably signal their parental care quality when male care is essential for offspring survival. We evaluated these predictions in a neotropical frog with biparental care, *Anomaloglossus beebei*. We examined: (i) female preference for male advertisement traits, (ii) importance of male care on offspring survival and (iii) the relationships between male attractiveness and male care, female care and opportunities for multiple mates. Our results indicate that females prefer longer calls and that male care significantly impacts hatching success and offspring survival. Consistent with both the good parent and the essential male care hypotheses, we found more attractive males provided higher quality paternal care. These findings suggest that females may gain direct fitness benefits by selecting males that produce longer advertisement calls.

INTRODUCTION

Sexual selection theory holds that the relative difference between the sexes in parental investment directs the pattern of mate choice (Trivers 1972; Andersson 1994). Members of the sex that invest more in offspring are the choosier sex because they become the limiting resource for members of the opposite sex (Williams 1966; Trivers 1972). The choosier sex commonly selects among potential mates based on secondary sex traits associated with fitness benefits to the chooser, including *direct material benefits* (e.g. parental care), *indirect benefits* to the chooser's offspring (e.g. good genes), or both (Iwasa & Pomiankowski 1999). The extent to which these traits signal reliable information has been examined primarily in studies of indirect benefits in species in which males invest relatively little in offspring and females are the choosier sex (reviewed in Andersson & Iwasa 1996). More recent studies have investigated mate signaling in species in which males provide significant direct benefits, especially in terms of parental care (Badyaev & Hill 2002; Dolby et al. 2005; Halupka & Borowiec 2006; Lindström et al. 2006; Mitchell et al. 2007; Oliver & Lobel 2012; Pizzolon et al. 2012). Specifically, these studies have examined whether a male's secondary sex traits are honest indicators of his parental care quality. Thus far these studies have produced conflicting results suggesting that the relationship between male attractiveness and paternal care quality is not necessarily straightforward.

Current mate signaling theory identifies a number of factors that could influence the reliability of a signal to indicate paternal care quality including: quality of maternal

care, opportunities for multiple matings, and the importance of paternal care to offspring survival (Table 1). Initial indicator models of mate selection focused on two central hypotheses that predict opposite relationships between male attractiveness and paternal investment. The *good parent hypothesis* predicts that certain secondary sex traits should be *positively* correlated with male parental quality; specifically, males with more extravagant traits are predicted to provide better care (Hoelzer 1989). In such situations females gain direct benefits by selecting a mate with good parental abilities based on traits that honestly reveal paternal care quality. In contrast, the *differential-allocations hypothesis* predicts that attractive males will provide less parental care because their mates are willing to increase their share so that offspring that inherit “attractive” genes will also be more likely to survive (Burley 1986; Sheldon 2000). When empirical tests of these two hypotheses produced inconsistent results (Linville et al. 1998; Qvarnström 1997; Mountjoy & Lemon 1997) theoreticians suggested this conflict may be resolved after considering the increase in fitness an advertiser may gain from multiple matings (Magrath & Komdeur 2003). This *trade-off hypothesis* suggests that if opportunities for extra-pair copulations are numerous, the gain in fitness from multiple matings would be high and secondary sex traits should not necessarily be honest predictors of paternal care (Kokko 1998). In essence, for mates that provide parental care, the reliability of their advertisement signal depends on the availability of multiple mates (Møller & Thornhill 1998). Most recently, a fourth hypothesis has been proposed to explain signal reliability in species in which males provide extensive care that is essential for offspring survival (Kelly & Alonzo 2009, 2010). This *essential male care hypothesis* predicts that when

offspring survival requires either obligate biparental care or male-only care, then male advertisement will always be a reliable indicator of male parental care (Kelly & Alonzo 2009, 2010).

Empirical tests of these hypotheses have been conducted primarily in birds with biparental care (Dolby et al. 2005; Halupka & Borowiec 2006; Mitchell et al. 2007) and in fish with male-only care (Lindström et al. 2006; Oliver & Lobel 2012; Pizzolon et al. 2012). The present study aims to differentiate among these theoretical models by examining mate selection and parental care in the biparental golden rocket frog (*Anomaloglossus beebei*). This neotropical frog exhibits a suite of characteristics that make it well-suited to test the predictions of each of the four hypotheses. First, the golden rocket frog, a bromeliad specialist, exhibits acoustically mediated mate selection in which males produce loud advertisement calls to attract females to potential oviposition sites (i.e., arboreal water pools or ‘phytotelmata’) within the males’ territories (Bourne et al. 2001; Pettitt et al. 2012). While the importance of acoustic signaling in mate selection has been well-documented in frogs (Gerhardt & Huber 2002), the extent to which advertisement calls signal parental care quality has not been studied. Second, this species exhibits biparental care with males providing extensive care including: egg and tadpole attendance, protection of eggs from desiccation and transport of tadpoles between phytotelmata, typically moving young to pools with fewer predators and more food (Bourne et al. 2001). Female care primarily consists of depositing unfertilized eggs that are cannibalized by developing tadpoles (Bourne et al. 2001). Because developing embryos and tadpoles are found exclusively in discrete phytotelmata of terrestrial tank

bromeliads, the quality of both male and female care can be readily observed and quantified. Third, males mate multiply and often attend to multiple egg clutches and tadpoles simultaneously (Bourne et al. 2001). In *A. beebei*, a male's opportunities for multiple mating can be quantified by measuring the amount of time spent producing advertisement calls and time spent in courtship. Lastly, the importance of male care on offspring survival can be measured using well-established techniques such as male removal experiments (Townsend et al. 1984; Juncá 1996; Vockenhuber et al. 2009; Cheng & Kam 2010).

The objective of the present study was to determine the extent to which traits of male golden rocket frog advertisement calls serve as an honest indicator to females of a male's parental quality. To differentiate among the theoretical models proposed to explain the relationship between male attractiveness and paternal quality, this study aimed: (i) to test for female preferences for male advertisement call traits to distinguish which call traits are used by females during mate selection, (ii) to determine the importance of male parental care on offspring survival, and (iii) to assess the relationships between call traits and paternal care quality, maternal care quality and multiple mating opportunities.

METHODS

A. Study site

Parental care, mating behavior and offspring survival in the golden rocket frog were studied from May-October 2009 and May-July 2010. Endemic to Guyana, South America, *A. beebei* is found only in Kaieteur National Park (Kok et al. 2006). We studied a free-ranging population in the park along the edge of a 455 m plateau near Kaieteur Falls (5°1'23"N, 59°28'52"W). Golden rocket frogs are commonly found in open glades near the falls where giant bromeliads are abundant and mist from the falls is continually present (Bourne et al. 2001). Breeding occurs year round, but peaks between mid-May to mid-August during the long rainy season (Bourne & Osborne 1978; Bourne et al. 2001).

B. Female playback tests

1. General testing procedures

Between May–July 2009 and June–July 2010, we captured gravid females which we identified by searching for active courtships between 0600-1000 h. Active courtships consisted of either a male producing courtship calls towards a female in the same phytotelm or a female in amplexus with a male. Females were kept in small plastic containers that were filled with approximately 25 ml of water from the phytotelm in which the female was collected. We released all females at the site of capture within six hours of being tested.

We tested female preference for call frequency, call rate and call duration using a two-choice playback design in a portable playback arena set up in the field. The circular playback arena was 1 m in diameter and made of a 2.5 cm high rigid foam bottom and 60

cm high sides made out of vinyl coated fencing and covered with black fabric that was acoustically-transparent but visually opaque. We placed two SME-AFS portable field speakers (Saul Mineroff Electronics, Elmont, NY) 180° apart and 1 m from the center of the arena such that they were 2 m apart facing each other (Fig. 4.1). We broadcast target signals from a Sony Vaio VGN-CR100 laptop computer (Sony Corp., New York, NY) using Adobe Audition v1.5 (Adobe Systems Inc., San Jose, CA). The sound pressure level (SPL in dB re 20 μ Pa, C-weighted, fast root-mean-square) of each target signal was equalized to 85 dB by placing the microphone of an Extech 407764 sound level meter (Extech Instruments, Waltham, MA) at the approximate position of a female's head while located at a central release site. This SPL is close to the mean call amplitude recorded in golden rocket frogs at a distance of 1 m (Pettitt et al. 2012). We observed behavioral responses using a JVC Everio GZ-MG130 harddrive camcorder (JVC Corp., Elmwood Park, NJ) that was mounted to a tent frame that was situated above the circular arena. The average \pm SD temperature during all playback tests was $26.2 \pm 2.1^\circ$ C (range: $23.0^\circ - 32.5^\circ$ C).

We started each test with a 1 min acclimation period immediately after a subject was placed in the release cage. We then broadcast alternating signals from the two speakers as a repeating loop during the entire duration of a test. Following 2 min of broadcasts, we released the female by remotely removing the lid of the release cage by pulling a string. We scored a positive response when a female came within 10 cm of the wall of the arena directly in front of one of the two speakers (see response zone in Fig. 4.1). A “no response” was recorded if a female failed to positively respond within 8 min.

We gave subjects a 5-10 min timeout between consecutive tests. We periodically alternated the stimuli between speakers to control for side bias. No such bias was detected. Following a switch in the relative positions of the two stimuli or a switch in test stimuli, we recalibrated the sound pressure levels.

2. Experimental design

We chose to test female preference for call frequency, call rate and call duration based on results of two previous studies looking at the potential for *A. beebei* advertisement call traits to function in mate selection. Using natural advertisement calls as broadcast stimuli, Bourne et al. (2001) found that female *A. beebei* ($N = 10$ females) preferred higher than average call rates and calls produced by larger males. In anurans, a larger overall body size is negatively correlated with fundamental frequency (Martin 1972; but also see Pettitt et al. 2012). Thus, we expected to find a female preference for higher call rates and lower call frequencies in *A. beebei*. Results of a recent study on call variation in 760 advertisement calls from 40 *A. beebei* males found call rate and call duration exhibited high amounts of within individual variation (Pettitt et al. in review). Following Gerhardt (1991), who concluded that call traits with high within-individual variation are expected to be under directional selection with females preferring extreme values, we expected *A. beebei* females to prefer higher call rates and longer call durations.

We conducted two-choice playback experiments (Gerhardt 1995) in which females were required to choose between two synthetic target signals. We used a custom-made sound synthesis program (written by J. J. Schwartz) to synthesize

individual pulses that we used to create target signals with call traits that fell within the natural range of variation. These call traits were based on temperature-corrected (24° C) mean values from previous recordings of 40 males from the local population (Pettitt et al. 2012). To test preferences for call frequency, we conducted a series of five two-choice tests ($N = 25$ females/test for a total of 125 tests). In the first four tests we paired a “standard call” with a frequency of 5.4 kHz (population mean) against alternatives that differed from the mean frequency by ± 1 and ± 2 standard deviations (SD) (i.e., 5.1 kHz, 5.25 kHz, 5.55 kHz and 5.7 kHz). The fifth test consisted of a pairing of the two extremes (i.e., 5.1 kHz vs 5.7 kHz). The population mean and SD values were based on the natural distribution of dominant frequency in the calls of 40 previously recorded males (Pettitt et al. 2012). Similarly, we conducted a series of five two-choice tests to examine preferences for call rate ($N = 25$ females/test for a total of 125 tests). In the first four test we paired a standard call with a call rate of 26 calls/min (population mean) against alternatives that differed from the mean rate by ± 1 and ± 2 SD (i.e., 16, 21, 31, and 36 calls/min). The fifth test consisted of a pairing of the two extremes (i.e., 16 vs 36 calls/min). To test preferences for call duration we created four target signals with 2, 3, 4, and 5 pulses/call and tested all pairings for a series of six two-choice tests ($N = 25$ females/test for a total of 150 tests).

C. Male removal experiment

To determine the importance of male parental care on offspring survival, we removed attendant males from their territories and thus their egg clutches. During May – July

2010 we documented the fate of these experimental clutches and compared these data to that of control clutches which had males attending their clutches throughout embryonic development. Because *A. beebei* males can attend multiple clutches at the same time, we included only one clutch per male in our statistical analyses to avoid pseudoreplication. In such cases, we included only the first clutch identified. We identified both experimental and control clutches first by locating an active courtship (i.e., males producing courtship calls directed toward a female within the same phytotelm) and then monitoring the courtship until successful fertilization. Following fertilization, we caught both males and females when possible and clipped a unique number of toes for identification. We excluded any clutches that were produced following a courtship that involved more than one male and one female in a phytotelm.

We surveyed experimental and control clutches daily (between 0600-0900 h or 1600-1800 h) until they hatched or failed, recording clutch size, condition of each egg, presence/absence of adults and presence/absence of predators. We categorized the fate of each egg as: (i) predation, (ii) fungal infection, (iii), desiccation, (iv) failure to develop, (v) hatched or (vi) unknown. Eggs were considered depredated if embryos were missing less than 11 days following fertilization. After 11 days, the earliest age of known hatching, the fate of missing embryos was categorized as unknown because we did not know if the embryos hatched or were depredated. We considered that eggs had died due to a fungal infection when we saw hyphae covering the eggs, desiccation when the eggs had shriveled and become dehydrated, and failed to develop when embryonic growth was suspended for more than one week. Although most eggs whose embryos failed to

develop eventually showed signs of fungal infection, we categorized their fate as failing to develop. We identified eggs that had successfully hatched when we directly observed the hatching or when we saw an egg with a missing embryo the same day as we saw a new small black tadpole in the same phytotelm.

D. Relationships among male call traits, mating effort and parental care quality

1. Acoustic recordings and analyses

Between May – October 2009 and May– July 2010, we recorded a total of 261 advertisement calls from 29 males (9 calls/male). Frogs were recorded in the morning from 0600 to 0900 hours (h) and in the late afternoon from 1600 to 1800 h. Sound recordings were made using a Marantz PMD670 solid-state recorder (44.1 kHz sample rate, 16-bit resolution; Marantz America, Inc., Mahwah, New Jersey, USA) 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, Connecticut, USA). The tip of the microphone was held 1 m \pm 10 cm from calling males during a recording session. At the end of a recording session, we captured the male when possible, took photos of his natural markings, measured his 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 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, Illinois, USA); temperatures ranged between 23.3° C and 28.0° C with a mean of 25.1° C.

We used the automatic recognition tool of Avisoft-SASLab Pro v1.5 (Avisoft Bioacoustics, Berlin) to quantify the following eight spectral and temporal traits for the first pulse and its subsequent interval for each recorded call — dominant frequency (kHz), pulse duration (ms; onset to offset), pulse interval (ms; offset to onset), pulse rate (pulses/sec; 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 FFT (fast Fourier transform) with a Hanning window. We temperature corrected call frequency which was positively correlated with air temperature (Pearson's $r = 0.487$, $P = 0.007$, $N = 29$). From these data, we determined the mean \pm SD for each individual male for all eight call traits.

2. Mating effort, paternal care quality, and maternal care quality

We monitored the mating and paternal care behaviors of the 29 males for which we had advertisement call recordings as well as the maternal care behaviors of their mates and hatching success. We collected field observations of behaviors (range: 20.0 – 25.4 h for each set of parents) using a JVC Everio GZ-MG130 harddrive camcorder (JVC Corp., Elmwood Park, NJ) mounted on a tripod and directed towards the phytotelm containing their offspring. Video recordings were taken between 0600 – 1100 h. In some cases (12 of 29 males; 41%) we could confirm the identities of fathers in the video recordings by

their natural markings. In cases where fathers could not be identified by natural markings, we assumed the males seen in the video recordings were fathers of the present offspring because males aggressively respond to other male territorial intruders (Bourne et al. 2001). Females were assumed to be mothers of offspring unless they were engaged in courtship with the territorial male. Males and females were distinguished by presence/absence of vocal sacs, vocal behavior, body size and throat color.

We analyzed the following male mating behaviors: (i) time spent producing advertisement calls by the territorial male and (ii) time engaged in courtship behaviors with a female. We also analyzed the following parental care behaviors: (i) paternal attendance (i.e., time spent by fathers within approximately 30 cm of phytotelmata containing offspring), (ii) time spent by fathers in territorial defense, and (iii) maternal attendance (time spent by mothers within approximately 30 cm of phytotelmata containing offspring). We calculated mating effort (sum of time spent calling or in courtship), paternal care quality (sum of time spent in attendance and territorial defense) and maternal care quality (sum of time spent in attendance) as percentages of the total time of video recordings. Mating behaviors and paternal attendance were not considered mutually exclusive. In other words, time spent by males that produced advertisement calls or engaged in courtship within 30 cm of the focal egg clutch were included in calculations of both mating effort and paternal care quality. For 25 of the 29 sets of parents, video recordings were taken when offspring were 2-20 days olds (eggs or recently hatched tadpoles). For the remaining 4 sets of parents, video recordings were taken when offspring were more than 1 month old. As a second measure of the

importance of male care on offspring survival, we also monitored hatching success for all 29 egg clutches.

E. Statistical Analyses

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.

We collected a total of 123 gravid females and conducted 453 individual phonotaxis tests. All females were used as subjects in more than one test. We excluded the results from 53 tests from analyses because female subjects failed to make a choice within 8 min. All tests had a sample size of $N = 25$ females. We used separate one-tailed binomial tests to test the null hypothesis that the number of females approaching the presumably more “attractive” stimulus (e.g., calls with longer durations, faster rates or lower frequencies) was not greater than the number approaching the presumably less “attractive” stimulus (e.g., calls with shorter durations, slower rates or higher frequencies).

We analyzed the impact of paternal care on hatching success rate, causes of offspring mortality and offspring survival. We used nonparametric Chi-squared tests to compare the differences in hatching success rate and the causes of offspring mortality between two treatments: eggs with fathers present ($N = 179$ eggs from 36 clutches) and eggs with fathers removed ($N = 179$ eggs from 40 clutches). We conducted standard survival analysis (Nur et al., 2004) to compare daily offspring mortality between treatments. Survival analysis is appropriate for this study because: (i) some of the data

were censored (i.e., the fates of some eggs were not known prior to the end of data collection), (ii) all clutches were checked daily and (iii) we knew the age of failure because we identified focal clutches at the time of oviposition. Knowing the exact age of clutches reduces the concerns of left truncation that are typical of survival analysis (Heisey et al., 2007). We generated Kaplan-Meier failure curves (Bland & Altman, 1998) for offspring with and without attending males and compared the functions between these treatments using a log-rank test (Bland & Altman 2004).

We used two-tailed Spearman's rank-order correlations to explore the relationships among male call traits and male care, female care and male mating effort. Because extensive collinearity existed among call traits, we used principal components analysis (PCA) to account for this interdependency. Prior to the PCA, we standardized individual mean values of call traits by generating standardized Z scores to equally weight traits measured in different units (e.g., kHz and sec). These standardized values were used as input variables in the PCA. We extracted all principal components with eigenvalues greater than 1.0 and used the corresponding principal component scores as input variables in the correlation analyses. To examine the relationship between male parental care and offspring survival, we used a two-tailed Spearman's rank-order correlation to examine the relationship between male care and percent of clutch hatched and we used a Mann-Whitney U test to test for rank differences in male care between males with successful (one or more eggs hatched) and unsuccessful clutches.

RESULTS

A. Female playback experiment

We tested preferences for call frequency, call rate and call duration using 123 gravid females ($N = 101$ in 2009, $N = 22$ in 2010). The mean (\pm SD) number of signal combinations tested per female was 3.3 ± 2.1 (range: 2 – 9). Subjects required on average 193 ± 110 sec to enter the response zone. Population preference functions for call frequency, call rate and call duration are shown overlaid on natural distributions of each call trait (Fig. 4.2). These distributions were generated from individual averages using recordings of advertisement calls ($N = 19$ calls/male) from 40 males from the same population (Pettitt et al. 2012). Females showed no preference across the range of natural variation for call frequency (Fig. 4.2a) or call rate (Fig. 4.2b). Females did prefer, however, longer calls over shorter calls more often than expected by chance in all paired combinations (Fig. 4.2c).

B. Male removal experiment

We removed parental males from 40 clutches ($N = 179$ eggs). The fate of these experimental clutches was compared with that of 36 control clutches ($N = 179$ eggs). Average initial clutch size for experimental clutches (4.5 ± 0.8 eggs) did not differ from control clutches (5.0 ± 1.2 eggs; Mann-Whitney U test: $U = 554.0$, $df = 1$, $p = 0.07$). Hatching success rate was higher for eggs with attendant males (22%, 40/179) compared to eggs with males removed (7%, 12/179; $\chi^2 = 17.6$, $df = 1$, $P < 0.001$). Eggs without attendant males were more likely to be depredated (48% (86/179) vs 31% (55/179), $\chi^2 =$

11.2, $df = 1$, $P = 0.001$) and fail due to desiccation (7% (12/179) vs 2% (3/179), $\chi^2 = 5.6$, $df = 1$, $P = 0.02$). There was no difference in mortality due to fungal infection for eggs without attendant males (24%, 43/179) compared to those with attendant males (18%, 33/179; $\chi^2 = 1.7$, $df = 1$, $P = 0.20$). Because only 3 of 358 eggs failed to develop, we did not statistically test for differences regarding this cause of mortality.

Survival functions were constructed for our two treatments. The Kaplan-Meier curves are presented in Figure 4.3 and show higher survival rates for offspring with males present. The log rank test for a difference between the functions was significant ($\chi^2 = 19.7$, $df = 1$, $P < 0.001$). The Kaplan-Meier curves indicate that the treatment differences became most marked at about seven days after oviposition.

C. Relationships among male call traits, mating effort and parental care quality

For any call trait to function in mate selection as an indicator of paternal care quality, females should exhibit a preference for that particular trait when all other traits are controlled. Because female *A. beebei* did not exhibit a preference for either call rate or call frequency, we limited our correlation analyses to call traits related to the length of an advertisement call. Our PCA extract three principal components with eigenvalues of more than 1.0 (Table 2). Principal component 2 (PC2) loaded most heavily on the two highly correlated call traits associated with advertisement call length: number of pulses per call and call duration (Pearson's $r = 0.946$, $P < 0.001$, $N = 29$). Using PC2 as our call trait input variable in our correlation analyses, we found PC2 to be positively correlated with paternal care quality ($r_s = 0.443$, $P = 0.016$, $N = 29$) and mating effort ($r_s = 0.386$, P

= 0.038, $N = 29$), but was not correlated with maternal care quality ($r_s = 0.082$, $P = 0.67$, $N = 29$). Paternal care quality was not correlated with the percent of clutch hatched ($r_s = 0.196$, $P = 0.32$, $N = 29$). While there was no difference in male care provided by males with successful and unsuccessful clutches ($U = 68.0$, $P = 0.17$, $N = 29$), the percentage of time spent in parental attendance and territorial defense by males with successful clutches was almost twice that spent by males with unsuccessful clutches (10.4% versus 5.7% respectively).

DISCUSSION

Results of the current study provide three new contributions to the understanding of the relationship between acoustic signals and parental care in the context of mate selection in *A. beebei*. First, call duration, but not call rate or call frequency, is likely to be used by female *A. beebei* when selecting a mate. In particular, females prefer longer calls over shorter calls more often than expected by chance. Second, the presence of attendant males, while not always essential to the survival of offspring, resulted in increased offspring survival rates and hatching rates. Third, male *A. beebei* advertisement call properties related to call length honestly indicate paternal care quality supporting the good parent and the essential male care hypotheses. Consequently, our findings suggest that females may gain direct fitness benefits by selecting males that produce longer advertisement calls (e.g., calls with more pulses) because these males provide higher quality parental care.

A. Female playback experiment

The importance of acoustic communication in anuran mate selection has been well-studied (Ryan 2001; Gerhardt & Huber 2002). Patterns of female preferences for call traits that exhibit high amounts of within individual variation, such as call rate and call duration, typically show directional selection for more costly traits (e.g., higher call rates, longer call durations). On the other hand, preferences for traits that exhibit little variation from signal to signal within individuals, such as call frequency, more commonly show either stabilizing or weakly directional selection (Gerhardt & Huber 2002). Given results from previous studies of within individual call variation and of female preferences for calls produced by small and large males (Bourne et al. 2001; Pettitt et al. in review), we predicted that call rate, call duration and call frequency have the potential to be used in mate selection in *A. beebei*.

Results of our female playback tests revealed that female *A. beebei* did prefer longer calls, but they showed no preferences for higher call rates or lower call frequencies. Finding highly directional preferences for longer calls in *A. beebei* is consistent with the generally held view that females prefer more costly or elaborate male traits (reviewed in Ryan & Keddy-Hector 1992; Andersson 1994). Based on this same argument, however, we should have also seen a preference for higher call rates. This lack of preference was especially surprising given the results of previous studies looking at the potential role call rate plays in mate selection in *A. beebei*. One potential explanation for this result is based on the presence of a closely related species, Kaiei's rocket frog

(*Anomaloglossus kaiei*), that lives sympatrically with *A. beebei*. This sister species produces a call that is similar to *A. beebei*'s short, pulsed advertisement call, but has a slightly slower pulse rate, a lower dominant frequency (average = 4.85 kHz compared to *A. beebei*'s 5.4 kHz) and a faster call rate (average = 74 calls/min, range: 49-101 compared to *A. beebei*'s 26 calls/min, range: 20-41; Kok et al., 2006; Pettitt et al. 2012). Given that selection should favor signal divergence among closely related sympatric species to maintain species integrity (Noor 1999), a lack of preference for higher call rates in female *A. beebei* may reflect a response to multiple selection pressures. Specifically, in cases where high-quality conspecifics resemble heterospecifics, female preferences for costly traits (e.g., high call rates) may result in heterospecific mating mistakes even though preferences for low cost traits (e.g., low call rates) may result in matings with low-quality males (Gerhardt 1982; Pfennig 1998; Ryan & Rand 1993). For example, when female southern spadefoot toads (*Spea multiplicata*) co-occur with plains spadefoot toads (*Spea bombifrons*), female *S. multiplicata* appear to pass over high-quality matings to ensure conspecific matings (Pfennig 2000). A similar trade-off in costs between mate selection with heterospecifics and low-quality males may be occurring in *A. beebei*.

We also expected to see a preference for lower call frequencies; a result we did not find in our playback experiments involving synthetic target signals. Patterns of preference based on differences in call frequency are much more variable than those based on call rate and call duration (Gerhardt & Huber 2002). Some studies report strong directional preferences (Ryan et al. 1992; Wollerman 1998), while others report

stabilizing or weakly directional preferences (Grafe 1997; Márquez & Bosch 1997) or no preference (Lopez & Narins 1991). In *A. beebei*, a lack of preference for lower call frequency may be a result of heterospecific mating avoidance as suggested to explain preference patterns associated with call rate. On the other hand, dominant frequency was not found to be correlated with snout-vent length, mass, nor body condition (i.e., size-independent body mass) in male *A. beebei* (Pettitt et al. 2012) making it difficult for females to use differences in the frequency of calls to evaluate a male's body size or potential quality. While our results suggest that call frequency may not play a role in mate-quality recognition, this call trait may be useful in species recognition. Future playback tests could evaluate the role call frequency may play in species recognition in areas where both *A. beebei* and *A. kaiei* co-occur.

B. Male removal experiment

Results of our male removal experiment show that attending *A. beebei* males contributed significantly to the survival of their clutches, but that their care was not essential to offspring survival (i.e., some offspring successfully hatched despite the removal of the attendant male). In particular, eggs with attendant males showed higher survival rates and higher hatching success compared to eggs without attendant males. These results are consistent with the findings of other anuran removal experiments in which offspring survival was compared between egg clutches with attendant adults and those without (Simon 1983; Townsend et al. 1984; Juncá 1996; Bourne 1998; Vockenhuber et al. 2009). A review by Crump (1995) identified potential functions of parental care in frogs

including reducing risks of predation, desiccation and fungal infections. In *A. beebei*, the presence of attendant males was found to decrease offspring mortality caused by predation and desiccation. While defensive behavior towards crabs (the most common embryonic predator of *A. beebei*) was never observed in adults, the presence of attendant males appears to reduce such predation risks. Similar anti-predator benefits attributable to parental care have been identified in other anurans including: *Hyalinobatrachium valerioi* (Vockenhuber et al. 2009), *Eleutherodactylus cooki* (Burrowes 2000), *Colostethus stephensi* (Juncá 1996), and *Hylophorbus rufescens* (Bickford 2004). Mortality due to desiccation is one of the most commonly suggested causes of terrestrial anuran offspring loss (Salthe & Mecham 1974; Taigen et al. 1984). Parental behaviors that are known to reduce desiccation risks include actively moistening eggs and transferring water from parent to egg across the male's ventral integument (Taigen et al. 1984; Townsend et al. 1984; Duellman & Trueb 1994). In *A. beebei*, males protect eggs from desiccation by squirting fluid from their cloacae to maintain moisture (Bourne et al. 2001). Although the presence of attending parents may reduce fungal infection in some frogs (Blommers-Schlösser 1975; Simon 1982), this does not appear to be the case for *A. beebei* which showed similar rates of fungal infection in eggs with and without attendant males.

A small percentage of *A. beebei* eggs without attendant males (7%, see Results) were still able to successfully hatch. This result indicates that paternal care is not essential to offspring survival. We suggest that this finding could be due to an increase in parental effort by an attending female. On one occasion during our daily surveys, one of

us (BAP) observed a female transporting a small black tadpole from its natal phytotelm; the first time a female *A. beebei* has been seen transporting young. This larva was one of four tadpoles, out of a clutch size of six, that successfully hatched despite the removal of the attending male. It is important to note that, although females appear to be capable of performing the care provided by males, males are not capable of providing unfertilized eggs for developing tadpoles, the primary feature of female *A. beebei* care.

C. Relationships among male call traits, mating effort and parental care quality

We found a positive correlation between the length of a call (i.e., principal component that corresponds to call duration and the number of pulses in a call) and male parental care quality. This result suggests that call length is an indicator of male parental care quality and may be used by females during mate selection to gain direct fitness benefits. This finding provides support for the good parent hypothesis (Hoelzer 1989) which proposes that females should benefit by evaluating advertisement traits that reliably signal parental quality. It follows that offspring that receive higher quality paternal care should have higher rates of survival. Although our male removal experiment results indicated that the presence of attendant males is important for offspring survival, we did not find any relationship between male parental care and hatching success. One possible reason for this lack of a relationship could be related to the high offspring mortality rates found in *A. beebei*. In both our male removal experiment and our correlation study we found that only 22% of eggs survived to hatching when an attendant male was present. Consequently, our sample size of 29 males and their associated offspring may have been

too small to detect a correlation between male care and hatching success even if such a relationship exists. Further empirical work is needed to more fully understand the extent to which paternal care quality impacts offspring survival in *A. beebei*.

Empirical evidence supporting the good parent hypothesis has been found in both birds with biparental care (Welling et al. 1997; Buchanan & Catchpole 2000; Penteriani et al. 2002; Dolby et al. 2005; but see Hadfield et al. 2006) and fish with male only care (Knapp & Kovach 1991; Lindström et al. 2006). These studies found paternal care quality to be positively correlated with various characteristics of acoustic signaling in birds including song rate (Welling et al. 1997; Dolby et al. 2005) and repertoire size (Buchanan & Catchpole 2000) and characteristics of courtship in fish including courtship rate (Knapp & Kovach 1991) and fanning behavior (Lindström et al. 2006). The use of care behaviors, such as fanning in fish, as a means to attract females is likely to occur primarily in species that care for multiple sets of offspring that overlap in time. Like most fish with parental care, many dendrobatids including the golden rocket frog care for multiple egg clutches simultaneously. While our study did not examine the extent to which males advertise paternal care quality through the use of male care behaviors, future studies of mate selection in *A. beebei* should investigate this possibility.

Findings of the current study do not support predictions of the differential allocations hypothesis (Burley 1986). This hypothesis predicts that attractive males will provide less parental care because their mates are willing to increase their share so that their offspring will inherit “good genes.” Support for the differential allocation hypothesis has come primarily from experimental studies involving biparental bird

species in which females paired with more attractive males provisioned nestlings more enabling males to decrease their provisioning efforts (Qvarnström 1997; Badyaev & Hill 2002; Johnsen et al. 2005). In the golden rocket frog, we found a positive correlation between attractiveness (measured in terms of call length) and male care quality and no correlation between call length and female care quality. While these results do not rule out the possibility that call length may provide some indication of genetic quality, it does not appear that females alter their parental care effort according to male attractiveness. We did observe anecdotal evidence of a female golden rocket frog compensating for reductions in male care (e.g., providing tadpole transport which is normally performed by males) after the attending male was removed. However, the results of our male removal experiment suggest that only *some* females may be willing and able to compensate fully for a decrease in male parental effort and that the impact of male care on offspring survival is significant. We speculate that female *A. beebei* may face a trade-off between parental effort in the form of attendance and tadpole transport and parental effort in the form of trophic egg provisioning and that only some females may be in good enough condition to provide both types of care.

The trade-off hypothesis proposes that the reliability of advertisement signals depends on the availability of multiple mates. This hypothesis predicts that when opportunities for matings are abundant, male traits should not necessarily be honest predictors of paternal care quality; males benefit more by increasing mating effort and decreasing parental care effort (Kokko 1998). In *A. beebei*, multiple egg clutches and tadpoles are often found within the territory of one male (Pettitt unpublished data)

suggesting that mating opportunities are numerous. According to the trade-off hypothesis, we would expect male attractiveness to be negatively correlated with paternal care effort and positively correlated with mating effort. However, we found a positive relationship between call length and both male care and male mating effort. We suggest that this apparent lack of conflict between mating and parental investments may be a consequence of the features of *A. beebei* breeding and paternal care. First, male *A. beebei* continue to breed at the sites of current offspring care. As a consequence, the conflicts between mating effort and parental care effort are likely to be minimal or nonexistent (Stiver & Alonzo 2009). Second, the primary form of parental care provided by males, offspring attendance, is “nondepreciable,” i.e., independent of offspring number (Clutton-Brock 1991). Such forms of care are considered to have lower costs because benefits to individual offspring do not decline with increasing numbers of offspring or sets of offspring. If parental care costs are low, males are able to invest more energy into advertising and mating. It is not surprising, therefore, that evidence supporting the trade-off hypothesis has been found in species in which males provide costly, depreciable care (e.g., chick provisioning) that negatively impacts mating effort (Halupka & Borowiec 2006; Mitchell et al. 2007).

The essential male care hypothesis considers the trade-offs between mating and paternal care effort under the particular condition in which male care is essential to offspring survival. This hypothesis proposes that, in cases of obligate biparental and male-only care, a male’s advertisement trait may signal some aspect of mate quality, but it should not trade-off with a male’s ability to provide parental care (Kelly & Alonzo

2009). Under such conditions, males are expected to reliably signal paternal care quality and females are expected to prefer such males because females cannot compensate for low quality or non-existent male care (Kelly & Alonzo 2009). Results of the current study are consistent with these theoretical expectations as male golden rocket frogs do reliably signal their parental care quality and, at least in some cases, females do not appear to compensate for non-existent male care. Comparable results were found in a fish with exclusive male care in which, similar to *A. beebei*, males attend overlapping clutches from multiple females (Pizzolon et al. 2012). In both cases conflicts between mating and parental care efforts may be minimal allowing males to allocate sufficient resources to both advertising and caring. While our study did reveal that male care is not always essential to offspring survival, based on results from our male removal experiment, we speculate that the ability of a female to compensate for low quality paternal care may depend on her current condition. Further research is needed to evaluate the ability of both male and female golden rocket frogs to assess and respond to changes in the parental care provided by their mates.

In conclusion, our study provided initial evidence in support of both the good parent and the essential male care hypotheses. This research is the first to evaluate the predictions of these four mate signaling hypotheses in a frog with biparental care. This species provides a unique opportunity to test the application of these hypotheses because of its unusual division of parental care. Unlike most biparental birds in which both males and females provide similar types of care (e.g., chick provisioning) during the same temporal intervals, male and female golden rocket frogs typically provide different forms

of care (e.g., attendance and tadpole transport versus trophic egg provisioning) to offspring at different developmental stages (Bourne et al. 2001; this study). We speculate that, under such conditions, honest signaling of paternal care quality benefits both males and females. If a male honestly indicates his parental care quality, females benefit by proportionally allocating their resources to offspring of males with high-quality parental abilities. Males benefit, on the other hand, because they must rely on their mates to provision the tadpoles in which they have already invested heavily. Our study offers the first insights into the role male *A. beebei* advertisement traits play in signaling paternal care quality. Additional evidence is needed to confirm our correlation results and to quantify the fitness benefits received due to female mate selection for these advertisement traits.

Table 4.1: The hypotheses addressing the relationship between male secondary sex traits and paternal care quality

Name	Hypothesis	Predictions
Good Parent	Trait signals quality of paternal care *(Hoelzer 1989)	Positive correlation between trait attractiveness and quality of male care
Differential Allocations	Trait signals good genes or attractiveness and female willing to increase her share of parental effort *(Burley 1986)	Negative correlation between trait attractiveness and quality of male care Positive correlation between trait attractiveness and quality of female care
Trade-Off	Trait signals good genes or attractiveness and is related to availability of additional mating opportunities *(Kokko 1998)	If males have multiple mating opportunities, trait attractiveness will be negatively correlated with quality of male care If males do not have multiple mating opportunities, trait attractiveness will be positively correlated with quality of male care
Essential Male Care	Trait signals quality of paternal care and male care is essential for offspring survival *(Kelly & Alonzo 2009)	If male care is essential for offspring survival, trait attractiveness will be positively correlated with quality of male care

Table 4.2: Results from a principal components analysis showing the factor loadings for all eight factors.

Call Property	PCA Factor		
	1	2	3
Pulses Per Call	0.038	0.427	-0.255
Call Duration	-0.028	0.463	-0.127
Call Interval	-0.292	-0.104	0.034
Call Rate	0.289	0.026	0.056
Pulse Duration	0.000	0.203	0.562
Pulse Interval	-0.293	0.011	-0.065
Pulse Rate	0.258	-0.159	-0.264
Dominant Frequency	0.105	0.031	0.467
Eigenvalue	2.993	2.101	1.443
Variance (%)	37.4	26.3	18.0
Cumulative % of variance	37.4	63.7	81.7

Factor loadings greater than 0.255 are highlighted in boldface type

Figure 4.1: Schematic diagram of the circular playback area (1 m diameter) used for female phonotaxis tests showing the position of the central release cage, the speakers and the response zones relative to the arena wall.

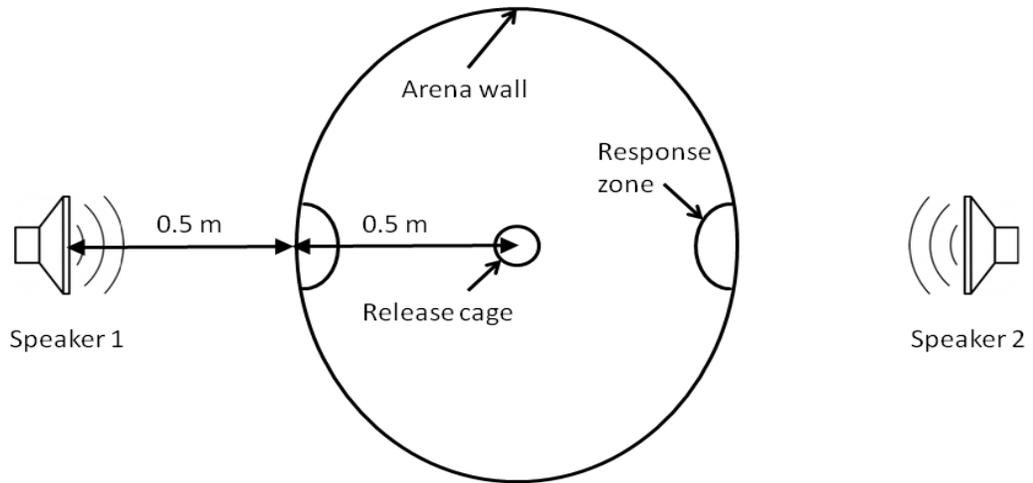


Figure 4.2: Histograms showing the distribution of call frequency (a), call rate (b) and call duration (c) using individual means from 40 males. Lines overlaid on histograms depict female preference functions showing the number of females that chose each alternative as a function of the call trait in two-choice discrimination tests. Lines join the two alternatives in each test; solid lines indicate a significant difference (two-tailed binomial; $P < 0.05$) and dashed lines indicate no significant difference (two-tailed binomial; $P > 0.05$).

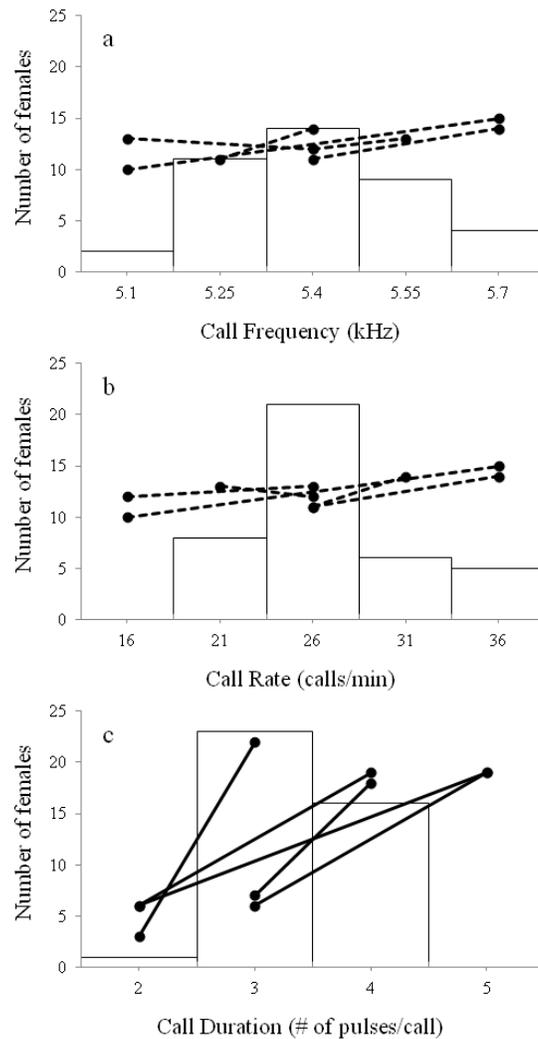
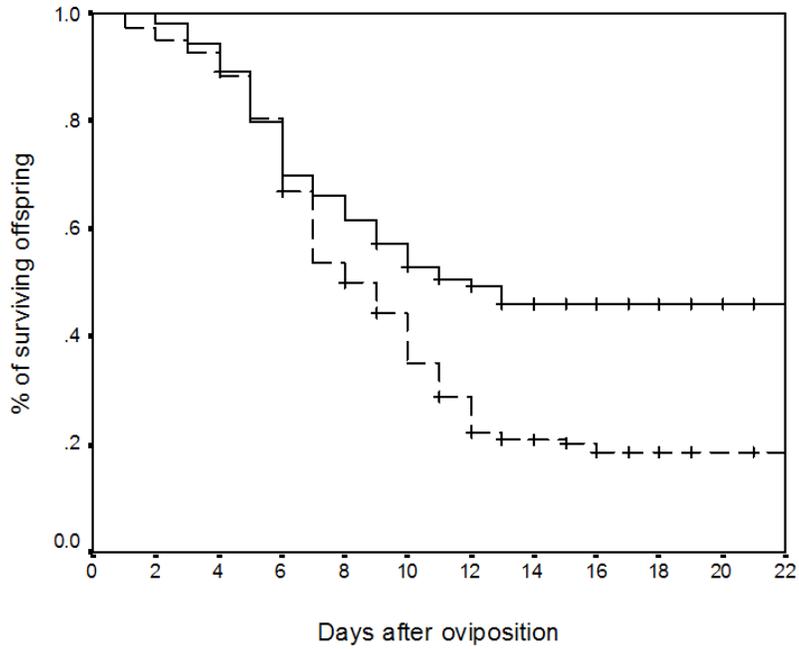


Figure 4.3: Survival functions for the time of the longest surviving offspring for egg clutches with an attendant male ($N = 36$; solid line) and egg clutches without an attendant male ($N = 40$; dashed line). The times of the censored data are indicated by + markers.



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