



Original Article

# Females prefer the calls of better fathers in a Neotropical frog with biparental care

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Male secondary sexual traits potentially function as indicators of direct or indirect fitness benefits to females. Direct benefits, such as paternal care, may be especially important to females in species with biparental care. In an experimental field study of the golden rocket frog (*Anomaloglossus beebei*), a Neotropical species with biparental care, we tested predictions from four hypotheses proposed to explain the evolutionary relationship between male secondary sexual traits and paternal care quality (the “good parent,” “differential allocation,” “trade-off,” and “essential male care” hypotheses). We examined: 1) the influence of paternal care on offspring survival, 2) the relationships between male calls and paternal care, maternal care, and opportunities for males to acquire multiple mates, and 3) female preferences for three acoustic properties of male advertisement calls. Our results reveal that paternal care positively impacts offspring survival, that males producing longer calls also provide higher-quality paternal care in the form of greater egg attendance and territory defense, and that females prefer longer calls. Females did not discriminate among potential mates based on differences in dominant frequency or call rate. These findings, which suggest male advertisement calls are indicators of potential direct benefits to females in the form of paternal care, are consistent with the good parent hypothesis and inconsistent with the trade-off, differential allocation, and essential male care hypotheses.

**Key words:** acoustic signals, direct benefits, honest signaling, mate choice, parental care, sexual selection

## INTRODUCTION

Empirical and theoretical work on mate signaling by males has identified numerous factors that influence how reliably signals indicate paternal care quality (Table 1), including the quality of maternal care, opportunities for multiple matings, and the contribution of paternal care to offspring survival (Burley 1986; Hoelzer 1989; Mountjoy and Lemon 1997; Qvarnström 1997; Kokko 1998; Linville et al. 1998; Sheldon 2000; Magrath and Komdeur 2003; Dolby et al. 2005; Halupka and Borowiec 2006; Lindström et al. 2006; Gowaty et al. 2007; Mitchell et al. 2007; Kelly and Alonzo 2009, 2010; Pizzolon et al. 2012; Oliver and Lobel 2013; Haaland et al. 2017). Early indicator models of mate selection focused on two central hypotheses that predict opposite relationships between male attractiveness and paternal investment. The “good parent hypothesis” (Hoelzer 1989) predicts that males are selected to honestly advertise the quality or quantity of care they are likely

to provide if paternal care influences offspring viability or fertility. Hence, males with more extravagant secondary sex traits are predicted to provide better parental care (Hoelzer 1989). In such situations, females gain direct benefits by selecting a mate with good parental qualities based on traits that honestly reveal paternal care quality. In contrast, the “differential allocation hypothesis” (Burley 1986; Sheldon 2000) predicts that attractive males provide less parental care because their mates are willing to increase their own parental effort, either to increase the survival of offspring that inherit genes conferring attractiveness (“positive differential allocation”; Haaland et al. 2017) or to compensate for the lack of paternal care that would otherwise reduce offspring survival (“negative differential allocation” or “reproductive compensation”; Gowaty et al. 2007; Haaland et al. 2017).

When empirical tests of the good parent and differential allocation hypotheses produced inconsistent results (Mountjoy and Lemon 1997; Qvarnström 1997; Linville et al. 1998), additional theory was developed suggesting this conflict could be resolved after considering the trade-off between parental effort and mating effort as well as the potential increase in fitness an advertiser may gain from multiple matings. This “trade-off hypothesis” (Magrath

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**Table 1**  
**Hypotheses and predictions**

Name	Hypothesis	Predictions
Good parent	Trait signals quality of paternal care.	Positive correlation between trait attractiveness and quality of male care.
Positive or negative differential allocation	Both: Trait signals good genes or attractiveness. Positive: Female increases share of parental effort to capitalize on benefits of mating with a high-quality male. Negative: Female increases share of parental effort to compensate for costs of mating with low-quality male.	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.	Negative correlation between trait attractiveness and quality of female care.
Essential male care	Trait signals quality of paternal care and male care is essential for offspring survival.	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. If male care is essential for offspring survival, trait attractiveness will be positively correlated with quality of male care.

and Komdeur 2003) predicts that if the gain in male fitness from multiple matings is high, then secondary sex traits should not necessarily be honest predictors of paternal care (Kokko 1998). In essence, for males that provide paternal care, the reliability of their sexual advertisement signal should vary inversely with the availability of multiple mates (Møller and 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. This “essential male care hypothesis” (Kelly and Alonzo 2009, 2010) predicts that when offspring survival requires either obligate biparental care or male-only care, then male advertisement should be a reliable indicator of male parental care. Unlike the previous hypotheses, this hypothesis assumes paternal care is absolutely required for offspring survival and that the allocation of resources to paternal care or obtaining mates is determined by which is the more fitness-limiting trait.

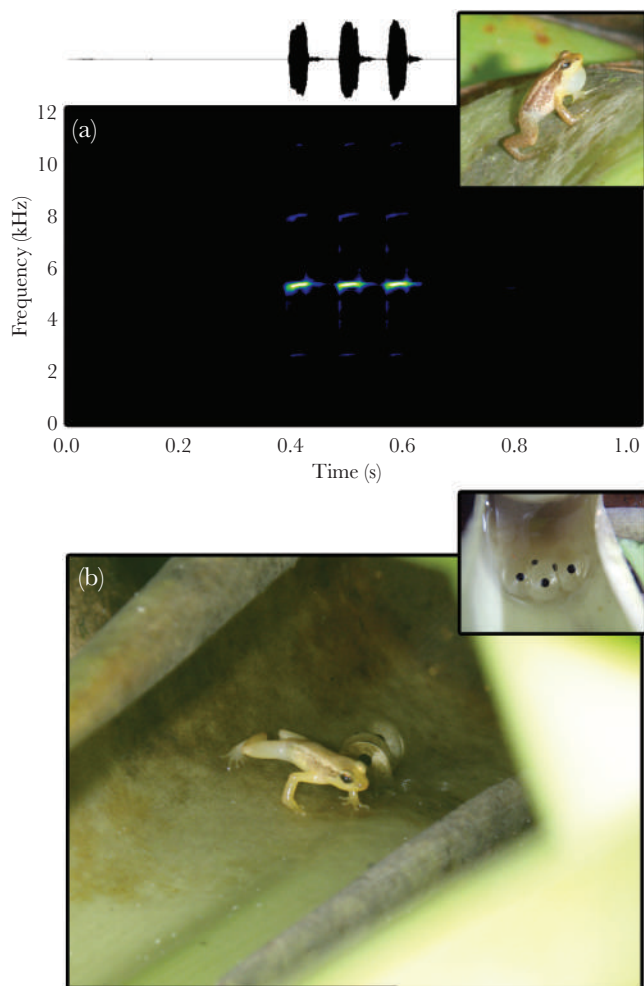
Empirical tests of these four alternative hypotheses have been conducted primarily in birds with biparental care (Dolby et al. 2005; Halupka and Borowiec 2006; Mitchell et al. 2007) and in fish with male-only care (Lindström et al. 2006; Pizzolon et al. 2012; Oliver and Lobel 2013). The extent to which male secondary sexual traits might function as indicators of parental care quality has not been studied in anuran amphibians (but see Ospina et al. 2017), although diverse parental care behaviors and the importance of acoustic signaling in mate selection are well documented in this group (Crump 1995; Gerhardt and Huber 2002; Wells 2007; Schulte et al., in press). Using a strong inference approach (Platt 1964) to differentiate among the four alternative hypotheses described above, we investigated parental care and mate selection in the golden rocket frog (*Anomaloglossus beebei*). Endemic to Guyana, South America, where it is found only in the Kaieteur National Park (Kok et al. 2006), the golden rocket frog exhibits acoustically mediated mate selection and biparental care. Males produce loud advertisement calls to attract females to potential oviposition sites in bromeliads (i.e., arboreal water pools or “phytotelmata”) within the males' territories (Figure 1a; Bourne et al. 2001; Pettitt et al. 2012, 2013, 2018; Tumulty 2018). Males also provide extensive paternal care, including egg and tadpole attendance (Figure 1b), protection of eggs from desiccation, and transport of tadpoles between phytotelmata, which typically involves moving offspring to pools with fewer predators and more

food (Bourne et al. 2001; Pettitt et al. 2018). Males mate multiply and often provide care for multiple egg clutches and tadpoles simultaneously (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, and the impact 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 and Kam 2010).

We conducted the following experiments and observations in the field to test predictions from the four alternative hypotheses proposed to explain the relationship between male attractiveness and paternal quality (Table 1). We experimentally removed males from attended egg clutches to determine the influence of paternal care on offspring survival through the early tadpole stage of development. Video and acoustic recordings were made to quantify the relationship between call properties and paternal care quality, maternal care quality, and multiple mating opportunities. Finally, we conducted two-alternative choice tests to investigate female preferences for acoustic properties of male advertisement calls. Together, data from these experiments and observations, which focused on parents of offspring in early developmental stages, are consistent with the good parent hypothesis and allowed us to reject the differential allocation, trade-off, and essential male care hypotheses.

## MATERIALS AND METHODS

All observations, collections, and experimental procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC, protocol #0912A75263) and were carried out under a Biodiversity Research Permit (#190509BR109) from the Guyana Environmental Protection Agency. Experiments and observations were conducted from May to October 2009 and May to July 2010 along the edge of a 455-m-high plateau near Kaieteur Falls (5°1'23"N, 59°28'52"W), where golden rocket frogs are commonly found in open glades 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 rainy season (Bourne et al. 2001).



**Figure 1**

Vocal and parental care behavior in golden rocket frogs. (a) Waveform (top) and spectrogram (bottom) showing a single three-pulse advertisement call. The inset shows a calling male sitting on a bromeliad leaf in his territory. (b) Photograph of a male golden rocket frog attending a clutch of three eggs in a phytotelm inside his territory. The inset shows a closeup of a separate clutch of five eggs.

## Influence of paternal care on offspring survival

### Male-removal experiment

To determine the extent to which paternal care influenced offspring survival, we removed attendant males from their territories and thus their egg clutches. We documented the fate of these experimental clutches and compared these data to those from control clutches that had males attending their clutches throughout embryonic development. Because males can attend multiple clutches at the same time (Bourne et al. 2001; Tumulty 2018), we included only the first clutch identified for each male in our statistical analyses to avoid pseudoreplication. We identified both experimental and control clutches first by locating an active courtship (i.e., a male directing courtship calls toward a nearby female on the same bromeliad) and then monitoring the courtship until successful fertilization. Following fertilizations, we caught both males and females when possible and gave them unique identifying marks by toe clipping following accepted guidelines for using amphibians in field research (HACC 2004).

We tracked the fate of experimental and control clutches daily (between 0600 and 0900 h or 1600 and 1800 h) until they hatched or failed by recording clutch size, condition of each egg, presence/absence of adults, and presence/absence of predators. Average initial clutch size for experimental clutches ( $4.5 \pm 0.8$  eggs) did not differ from control clutches ( $5.0 \pm 1.2$  eggs;  $U = 554.0$ ,  $P = 0.07$ ). We categorized the fate of each egg as: 1) hatched, 2) predated, 3), desiccated, 4) infected with fungus, 5) failed to develop, or 6) unknown. We identified eggs that had successfully hatched when we directly observed the hatching or when we observed on the same day an egg with a missing embryo and a new, small tadpole in the same phytotelm. Eggs were considered predated if embryos were missing earlier than 11 days following fertilization. We considered eggs to have died due to desiccation when the eggs had shriveled and become dehydrated, and due to a fungal infection when we saw hyphae covering the eggs. Eggs were considered to have failed to develop when embryonic growth was suspended for more than 1 week. Although most eggs with embryos that failed to develop eventually showed signs of fungal infection, we categorized their fate as failing to develop. After 11 days, the earliest age of known hatching (Pettitt 2012), the fate of missing embryos was categorized as unknown because we did not know if the embryos hatched or were predated.

### Statistical analysis

We removed attending males from 40 clutches ( $N = 40$ ) and compared the fate of these experimental clutches to that of 36 control clutches ( $N = 36$ ). The unit of statistical analysis was each individual clutch ( $N = 76$  clutches total). (A total of 358 eggs—179 eggs in each treatment—were observed across all 76 clutches.) We did not statistically analyze the data using individual egg as the experimental unit because eggs were not independent of clutches, but we also report data for all eggs summed across clutches. Nonparametric Mann–Whitney  $U$  tests were used to compare the differences between the two treatments in the percent of clutch hatched and the causes of offspring mortality. We conducted standard survival analysis (Nur et al. 2004) to compare between treatments the daily offspring mortality of the longest surviving offspring within a clutch. Survival analysis is appropriate for this study because: 1) some of the data were censored (i.e., the fates of some eggs were not known prior to the end of data collection), 2) all clutches were checked daily, and 3) 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 survival curves for offspring with and without attending males and compared the functions between these treatments using a log-rank test (Bland and Altman 2004). These and all other statistical analyses described below were conducted using SPSS v12.0 (SPSS, Inc., Chicago, IL), and we used a significance criterion of  $\alpha = 0.05$  for all statistical tests unless otherwise indicated.

## Relationships among calls, parental care, and mating opportunities

### Quantifying acoustic properties of calls

The vocal repertoire of male golden rocket frogs (Pettitt et al. 2012) and the patterns and sources of individual variation in advertisement calls (Pettitt et al. 2013) have been described previously. The advertisement call consists of a brief, rapid series of short, similar pulses with a dominant spectral peak near 5.4 kHz (“dee-dee-dee”;

Figure 1a). We recorded a total of 261 advertisement calls from 29 males (9 calls/male). Frogs were recorded in the morning from 0600 to 0900 h and in the late afternoon from 1600 to 1800 h, when males are most actively calling (Bourne et al. 2001). Sound recordings were made using a Marantz PMD670 solid-state recorder (44.1 kHz sample rate, 16-bit resolution; Marantz America, Inc., Mahwah, NJ) and a handheld Sennheiser ME66 directional microphone (Sennheiser Electronic Corporation, Old Lyme, CT). The tip of the microphone was held at a distance of  $1.0 \pm 0.1$  m 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 gave him a unique identification mark using toe clipping. 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, IL); 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, Germany) 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/s; determined as one/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 (s; offset of one call to the onset of the next call), call rate (calls/min), and pulses per call. We selected the first pulse for these analyses because effect sizes for differences in pulse characteristics within a call are small, indicating that analyses of first pulses adequately capture variation among males (Pettitt et al. 2012). We generated spectrograms by applying a 512-point FFT (fast Fourier transform) with a Hanning window. Following Pettitt et al. (2012), we temperature corrected call frequency, which was positively correlated with air temperature (Pearson's  $r = 0.487$ ,  $P = 0.007$ ,  $N = 29$ ), to a common temperature of 24 °C. Other call properties were not correlated with temperature. From these data, we determined the mean  $\pm$  SD for each individual male for all eight call traits. The grand means of call properties from these analyses were similar (within one SD) of those from other studies of golden rocket frogs based on analyses of larger samples of calls/individual (Pettitt et al. 2013; Tumulty 2018).

### Quantifying paternal care, maternal care, and mating opportunities

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 the hatching success of their clutches. We made field observations of behaviors (range: 20.0–25.4 h for each set of parents) using a JVC Everio GZ-MG130 hard drive camcorder (JVC Corp., Elmwood Park, NJ) mounted on a tripod and directed towards the phytotelm containing their offspring. Videos were taken between 0600 and 1100 h. Of the 29 clutches followed, 5 clutches had no males observed, 20 clutches had one male observed, and 4 clutches had two males observed. Of the 28 males observed, we could confirm 12 males as fathers via natural markings, 3 males as not the father via natural markings, and 13 males we could not identify via natural markings. In 6 of these 13 cases, in which 2 males were present, we assumed the male initially found in the territory and exhibiting

territorial behaviors (e.g., producing territorial calls) was the father. In the remaining seven cases, in which only one male was present, we assumed the males seen in the video recordings were fathers of the present offspring. We believe these assumptions are valid because males, in general, exhibit remarkably high site fidelity and aggressively exclude other males from their territories (Bourne et al. 2001; Pettitt et al. 2012; Tumulty 2018). Females were assumed to be mothers of offspring unless they were engaged in courtship with the territorial male. This assumption is also valid because courtship between the parents of a developing clutch was never observed in this study and occurs only rarely (Bourne et al. 2001). Note that statistical outcomes were qualitatively similar if females in courtship were not excluded from these analyses. Males and females were distinguished by the presence/absence of vocal sacs, vocal behavior, body size, and throat color.

To quantify paternal and maternal care, we analyzed the following parental care behaviors: 1) offspring attendance (i.e., time spent by fathers within approximately 30 cm of phytotelmata containing offspring), 2) time spent by fathers in territorial defense, and 3) maternal attendance (time spent by mothers within approximately 30 cm of phytotelmata containing offspring). We analyzed offspring attendance because it is the most common and one of the most easily observed and quantified parental care behaviors exhibited by male golden rocket frogs (Bourne et al. 2001). While males also moisten eggs to prevent desiccation, we could not directly observe this behavior from our video recordings; however, we note that time spent preventing desiccation is included in our measure of egg attendance, as males need to be within 30 cm of the clutch while engaging in desiccation prevention. Tadpole transport was never observed during our video recordings, so we could not include it as an additional metric of parental care. We chose to assign male territorial behavior to parental effort because males in a related frog (*Allobates femoralis*, Aromobatidae) that take over the territory of a rival can become cannibals that consume all of the previous territory holder's developing clutches (Ringler et al. 2017). Hence, our inclusion of territorial defense as a metric of paternal care conforms with previous operational definitions of parental care, such as those of Blumer (1979, p. 149: “non-gametic contributions that directly or indirectly contribute to the survival and reproductive success of the offspring”) and Stahlschmidt (2011, p. 1: “any non-genetic contribution by a parent that appears likely to increase the fitness of its offspring”).

In frogs, mating opportunities are often strongly and directly related to a male's mating effort, as measured, for example, by the time devoted to attending choruses and calling to attract mates (e.g., Pröhl 2003; Friedl and Klump 2005; Ospina et al. 2017; reviewed in Wells 2007). Therefore, we used mating effort as a proxy for mating opportunities. To quantify mating effort, we analyzed the following male mating behaviors: 1) time spent producing advertisement calls and 2) time engaged in courtship behaviors with a female. We calculated paternal care quality (sum of time spent in attendance and territorial defense), maternal care quality (sum of time spent in attendance), and mating effort (sum of time spent calling or in courtship) as percentages of the total time of video recordings. Mating behaviors and egg attendance were not considered mutually exclusive, such that time spent by males that produced advertisement calls or engaged in courtship within 30 cm of the focal egg clutch was included in calculations of both mating effort and paternal care quality. As a cautionary note, we point out that the metrics we were able to examine in this field study probably do not capture all of the relevant variation in parental and

mating behaviors. Nevertheless, we believe they represent an important first step in quantifying these behaviors and understanding how they influence reproductive strategies in golden rocket frogs.

### Statistical analysis

We used two-tailed Spearman's rank-order correlations to examine the relationships among call properties and paternal care, maternal care, and male mating effort. Because extensive collinearity existed among call traits, we used principal component analysis (PCA) to account for this interdependence. 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 s). These standardized values were used as input variables in the PCA. We extracted all principal components with eigenvalues greater than 1.0. Using results from our female playback experiments (see below), we identified any extracted principal components that loaded most heavily on call traits likely to function in mate selection (i.e., those preferred by females). We used the corresponding principal component scores as input variables in the correlation analyses. We report those associations that remained significant after adjustment for multiple testing using the Benjamini–Hochberg correction with a False Discovery Rate of 10% (Benjamini and Hochberg 1995). To examine the relationship between paternal 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.

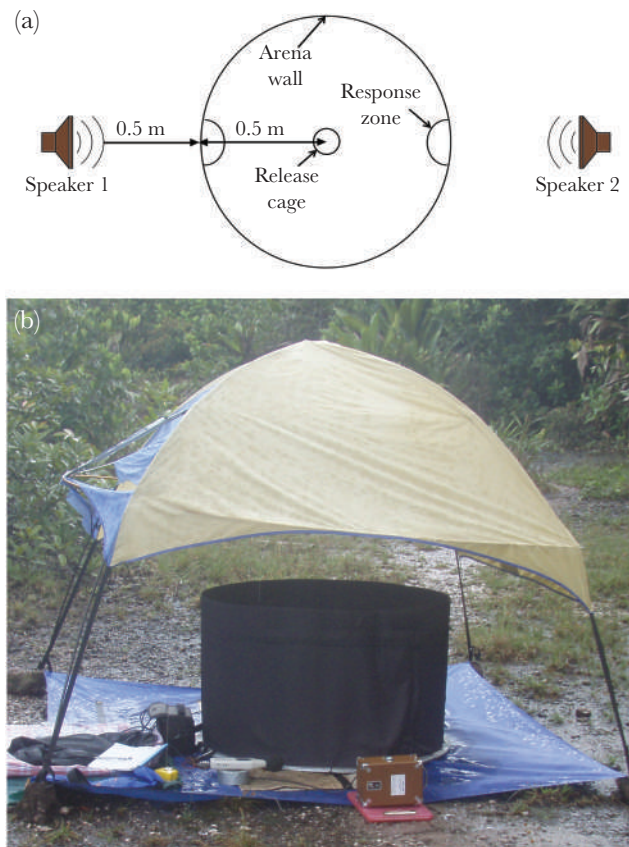
## Female preferences for male calls

### Two-alternative choice experiments

Using female phonotaxis as a behavioral assay, we evaluated mate choice preferences in three series of two-alternative choice tests (Gerhardt 1995). Females were required to choose between two synthetic target signals that differed in either spectral frequency (Series 1), call rate (Series 2), or call duration (Series 3), but were identical in all other respects. We used a custom-made sound synthesis program (written by J. J. Schwartz) to synthesize individual pulses, which we used to create target signals with call properties that fell within the natural range of variation. Values of call properties were based on temperature-corrected (24 °C) mean values from previous recordings of 40 males from the local population (Pettitt et al. 2012). Series 1 consisted of five two-alternative choice tests that examined female preferences for spectral frequency ( $N = 25$  females/test for a total of 125 tests). In the first four tests of this series, we paired a “standard call” with a dominant frequency of 5.4 kHz (population mean) against alternatives that differed from the mean dominant frequency by  $\pm 1$  SD and  $\pm 2$  SD (i.e., 5.1, 5.25, 5.55, and 5.7 kHz). The fifth test consisted of pairing the two extremes ( $+2$  SD versus  $-2$  SD) against each other (i.e., 5.1 kHz vs. 5.7 kHz). Stimulus calls in this series consisted of three pulses (population mean) and were delivered at rates of 26 call/min (population mean). In Series 2, we conducted five two-alternative choice tests to examine preferences for call rate ( $N = 25$  females/test for a total of 125 tests). In the first four tests, we paired a standard call with a call rate of 26 calls/min (population mean) against alternatives that differed from the mean rate by  $\pm 1$  SD and  $\pm 2$  SD (i.e., 16, 21, 31, and 36 calls/min). The fifth test again consisted of pairing the two extremes ( $+2$  SD vs.  $-2$  SD) against each other (i.e.,

16 vs. 36 calls/min). Stimulus calls in this series consisted of three pulses (population mean) and had dominant frequencies of 5.4 kHz (population mean). In Series 3, we tested preferences for call duration using four stimulus calls with 2, 3, 4, or 5 pulses/call. We tested all possible pairings of these stimuli for a series of six two-alternative choice tests ( $N = 25$  females/test for a total of 150 tests). Stimulus calls in this series had dominant frequencies of 5.4 kHz (population mean) and were delivered at rates of 26 call/min (population mean). Across all tests, the pulses in each call were 40 ms in duration and were separated by a silent interval of 50 ms.

Tests were conducted using gravid females as subjects. We identified gravid females by searching for active courtships between 0600 and 1000 h. Active courtships consisted of either a male directing courtship calls toward a female in the same phytotelm or a female in amplexus with a male. Females were housed in small plastic containers that were filled with approximately 25 mL of water from the phytotelm in or near which the female was collected. We released all females at the site of capture within 6 h of being tested after giving them unique identifying marks by toe clipping. We tested subjects in a portable test arena that we set up in the field (see Figure 2). The test arena was circular (1 m diameter) and constructed from 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



**Figure 2** Apparatus for testing female choice in the field. (a) Schematic diagram and (b) photograph of the circular playback area (1 m diameter) used for female phonotaxis tests. The diagram in (a) shows the position of the central release cage, the speakers, and the response zones relative to the arena wall.

and 1 m from the center of the arena such that they were 2 m apart facing each other. 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  $\mu$ Pa, C-weighted, fast root-mean-square) of each target signal was calibrated to 85 dB SPL 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 positioned the arena at a location sufficiently far from calling males so that our stimuli had SPLs in the test arena that exceeded those of ambient calls in the surrounding habitat by at least 20 dB. We observed behavioral responses using a JVC Everio GZ-MG130 hard drive camcorder (JVC Corp., Elmwood Park, NJ) that was mounted to a tent frame situated above the test arena. The mean  $\pm$  SD and median temperatures during all playback tests were  $26.2 \pm 2.1$  °C and 25.9 °C, respectively (range: 23.0–32.5 °C). We started each test with a 1-min acclimation period immediately after a subject was placed in a release cage in the center of the test arena. We then broadcast alternating signals from the two speakers as a repeating loop during the entire duration of a test. In Series 1 (spectral frequency) and Series 3 (call duration), the two alternative stimulus calls alternated in time such that an equal period of silence followed each call; in Series 2 (call rate), the two sequences of alternative calls started in alternation but thereafter had call timing relationships determined by their respective call rates. Following 2 min of broadcasts, we released the female by remotely removing the lid of the release cage. We scored a choice when a female approached to within 10 cm of the arena wall directly in front of one of the two speakers (see response zone in Figure 2a). A “no response” was recorded if a female failed to exhibit a choice within 8 min. We gave subjects a 5–10 min timeout between consecutive tests. We periodically (approximately every 10 tests) switched the stimuli between speakers to control for side bias. No such bias was detected. Following each switch in the relative positions of the two stimuli or a switch in test stimuli, we re-calibrated sound pressure levels.

### Statistical analysis

We collected a total of 123 gravid females ( $N = 101$  in 2009,  $N = 22$  in 2010) and conducted 453 individual phonotaxis tests. All females were used as subjects in more than one test (mean  $\pm$  SD:  $3 \pm 2$  tests/female, range: 2–9 tests/female), but each subject was tested only once for a specific pairing of two stimuli. Our final analyses do not include data from 53 tests in which subjects failed to make a choice within 8 min. All tests had a sample size of  $N = 25$ . Following standard procedures (Gerhardt 1995), we used two-tailed binomial tests to test the null hypothesis that the proportion of subjects choosing each paired stimulus did not differ from 0.5.

## RESULTS

### Influence of paternal care on offspring survival

The paternal care provided by attending males had positive effects on offspring survival: the percent of clutch hatched was significantly greater when males were present (mean  $\pm$  SD =  $20.33\% \pm 28.48\%$ ) compared to clutches with males removed (mean =  $6.96\% \pm 15.61\%$ ) ( $U = 557$ ,  $P = 0.04$ ; Figure 3a). Across clutches, hatching success rate was low whether attendant males were

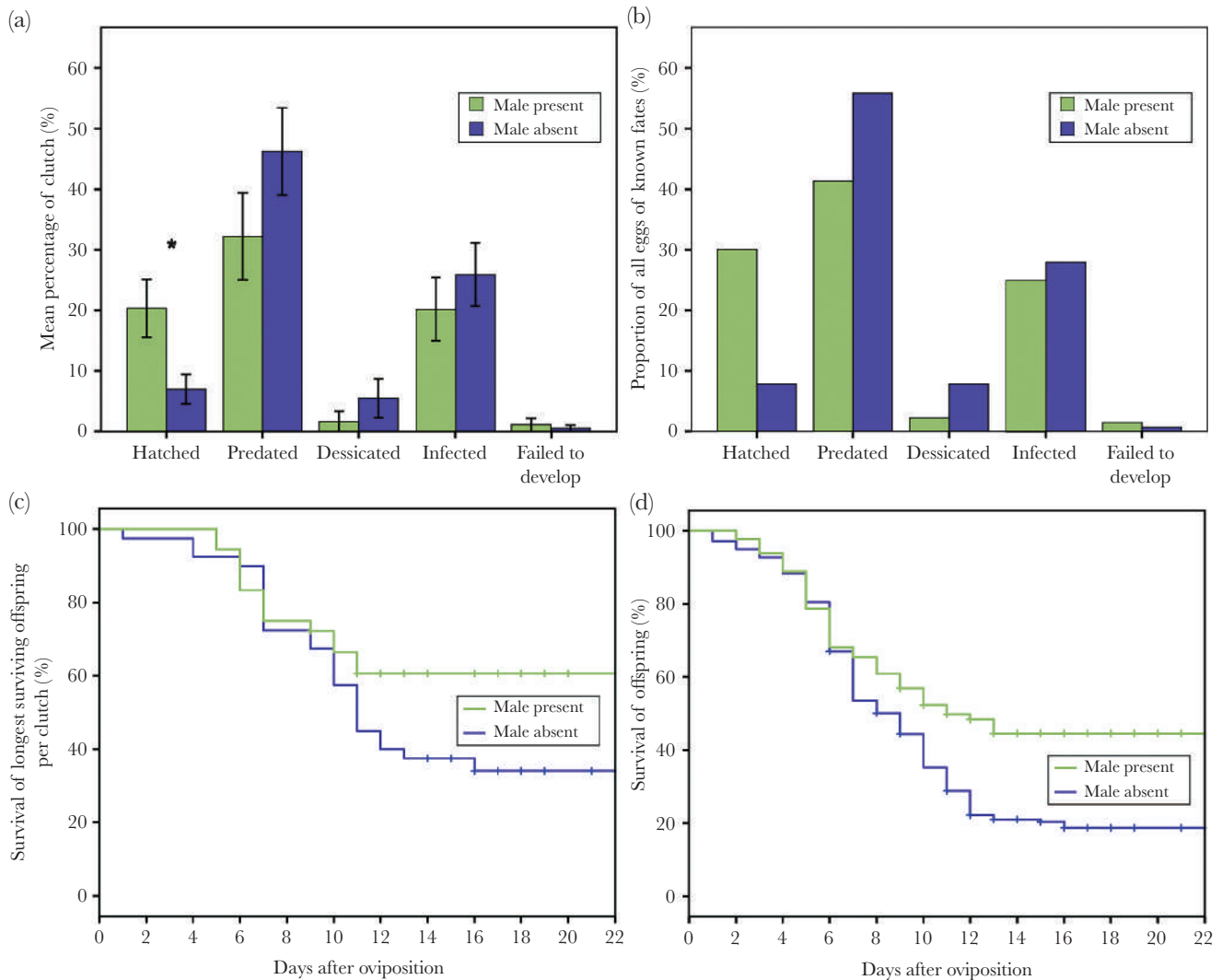
present or removed, but was nearly 4-fold higher for eggs with attendant males present (30%, 40/133) compared to eggs with males removed (8%, 12/154; Figure 3b). Notably, some eggs still hatched successfully when males were absent. Consistent with these general findings, survival functions showed a nonsignificant trend towards higher survival rates through time when using data for the longest surviving offspring within a clutch ( $\chi^2 = 3.1$ ,  $df = 1$ ,  $P = 0.078$ ; Figure 3c). A similar overall trend was apparent when considering all eggs (Figure 3d). Together, these data suggest offspring survival began to diverge between the male-present and male-absent treatments approximately 7–12 days after oviposition. The causes of offspring mortality did not differ between our treatments when considering clutches that were predated ( $U = 603$ ,  $P = 0.18$ ), desiccated ( $U = 686$ ,  $P = 0.35$ ), or infected with fungus ( $U = 653$ ,  $P = 0.44$ ; Figure 3a). Across clutches, however, there was a trend for eggs to be predated (56% [86/154] vs. 41% [55/133]) and to become desiccated (8% [12/154] vs. 2% [3/133]) at higher rates when the attendant male was absent versus present, whereas the rates of fungal infection were more similar when males were present (25% [33/133]) or absent (28% [43/154]) (Figure 3b). Because only 3 of 358 eggs failed to develop, we did not statistically test for differences regarding this cause of mortality.

### Relationships among calls, parental care, and mating opportunities

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 golden rocket frogs did not exhibit preferences based on either dominant frequency or call rate, but preferred longer duration calls (see below), we limited our correlation analyses to call traits related to the length of an advertisement call. Our PCA extracted three principal components with eigenvalues greater than 1.0 (Table 2). Principal component 2 (PC2) loaded most heavily on two call properties associated with advertisement call length, the number of pulses per call and call duration, which were strongly, positively correlated (Pearson's  $r = 0.946$ ,  $P < 0.001$ ,  $N = 29$ ). PC2 was 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 it was not correlated with maternal care quality ( $r_s = 0.082$ ,  $P = 0.67$ ,  $N = 29$ ). Paternal care quality was not significantly correlated with the percent of clutch hatched ( $r_s = 0.221$ ,  $P = 0.25$ ,  $N = 29$ ). The percentage of time spent in parental attendance and territorial defense by males with successful clutches was nearly twice that spent by males with unsuccessful clutches (10.4% vs. 5.7% respectively); however, there was no statistically significant difference in male care provided by males with successful and unsuccessful clutches ( $U = 68.0$ ,  $P = 0.17$ ,  $N = 29$ ).

### Female preferences for male calls

Females preferred relatively longer calls composed of a greater number of pulses but did not discriminate between calls differing in dominant frequency or call rate (Figure 4). Across all two-alternative choice tests in Series 1 (dominant frequency; Figure 4a) and Series 2 (call rate; Figure 4b), the proportion of subjects choosing the standard and alternative calls did not differ significantly from 0.5. In Series 3, the proportion of females choosing the longer call alternative was significantly greater than 0.5 in all six pairwise test combinations (Figure 4c). Across all 400 phonotaxis tests, subjects required, on average,  $193 \pm 110$  s to enter the response zone.



**Figure 3** Paternal care increases offspring survival. (a, b) Hatching success rates and the fate of experimental (attendant male removed) and control (attendant male present) (a) clutches and (b) individual eggs of known fates. Data for (a) clutches depict the mean  $\pm$  SD percentage of clutches and data for (b) eggs show the proportions of all eggs of known fates. (c, d) Kaplan–Meier survival curves using data from (c) the longest surviving offspring per egg clutch or (d) all eggs from all clutches. Green lines represent offspring with an attendant male (male present;  $N = 36$  clutches) and blue lines represent offspring without an attendant male (male absent;  $N = 40$  clutches). The times of the censored data are indicated by + markers. \* $P < 0.05$ .

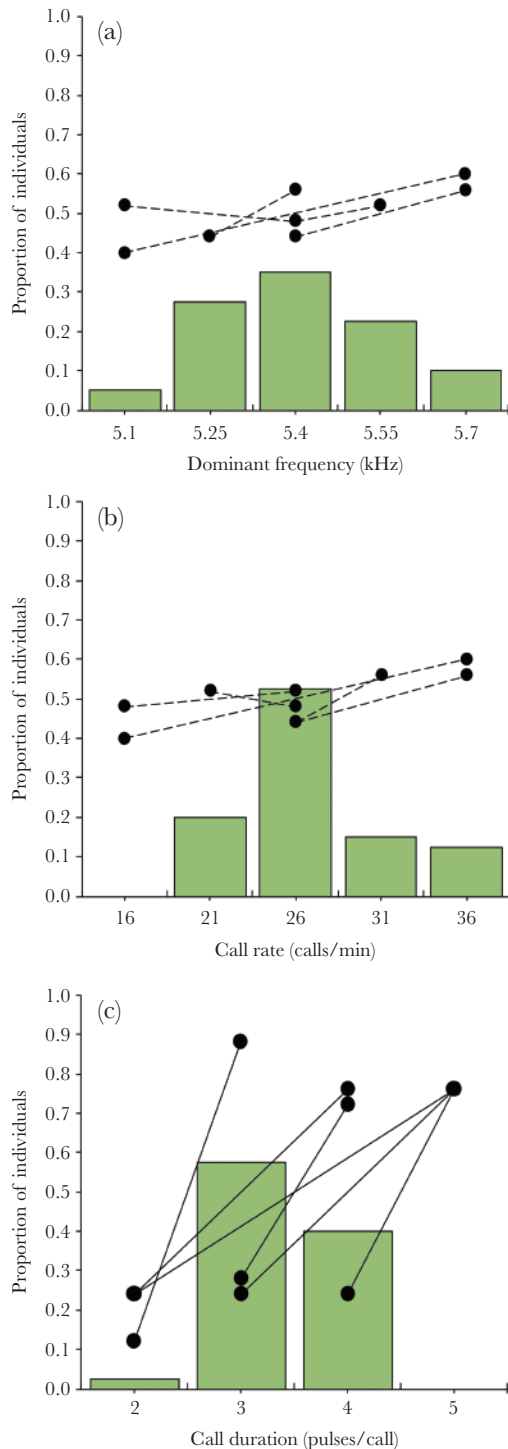
**Table 2** PCA showing the factor loadings for all eight call properties

Call property	PCA factor		
	1	2	3
Pulses per call	0.038	<b>0.427</b>	-0.255
Call duration	-0.028	<b>0.463</b>	-0.127
Call interval	<b>-0.292</b>	-0.104	0.034
Call rate	<b>0.289</b>	0.026	0.056
Pulse duration	0.000	0.203	<b>0.562</b>
Pulse interval	<b>-0.293</b>	0.011	-0.065
Pulse rate	<b>0.258</b>	-0.159	<b>-0.264</b>
Dominant frequency	0.105	0.031	<b>0.467</b>
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.

**DISCUSSION**

The main results of the present study can be summarized as follows. First, the presence of attendant males, while not always essential to the survival of offspring at the initial stages of development, resulted in higher hatching rates and likely higher offspring survival rates. Second, call duration was correlated with both the quality of the paternal care a male provided and his mating effort, but not the quality of maternal care provided by his mate. Finally, females preferred longer calls but did not discriminate between calls based on differences in dominant frequency and call rate. Together, these findings indicate that attending males make significant contributions to parental care that directly benefit females, that males vary in the quality of care they provide, that this variability is correlated with acoustic properties of male advertisement calls, and that females prefer calls indicative of higher-quality paternal care. Hence, females potentially gain direct fitness benefits in the form of paternal



**Figure 4** Females prefer longer calls with more pulses. Histograms showing the distributions of (a) dominant frequency, (b) call rate, and (c) call duration in our study population (drawn from data in Pettitt et al. 2012). These distributions were generated from individual means for each acoustic property using recordings of advertisement calls ( $N = 19$  calls/male) from 40 males. Lines overlaid on histograms depict preference functions showing the proportion of subjects ( $N = 25$  females) that chose each alternative as a function of the call trait in two-alternative choice tests. Lines join the two alternatives in each two-alternative choice test; solid lines indicate a significant difference (two-tailed binomial;  $P < 0.05$ ) and dashed lines indicate no significant difference (two-tailed binomial;  $P > 0.05$ ).

care of offspring by selectively mating with males that produce longer advertisement calls.

**Mate choice and parental care**

Our results are more broadly consistent with predictions of the good parent hypothesis than with those of the differential allocation, trade-off, and essential male care hypotheses (Table 1). Consistent with the good parent hypothesis (Hoelzer 1989), we found direct support for the prediction that females evaluate advertisement traits that reliably signal the quality of paternal care and indirect support for the prediction that offspring receiving higher-quality paternal care have higher rates of survival. Call length was positively correlated with our measures of paternal care quality, and as predicted, females exhibited directional preferences for longer calls. Our male-removal experiment indicated that the presence of attendant males is important, though not essential, for hatching success and likely offspring survival. While the correlation between paternal care quality and the percent of clutch hatched was positive ( $r_s = 0.221$ ), as expected, it was not statistically significant. This correlation is important to note for two interrelated reasons. First, the lack of statistical significance may stem from the generally low rates of offspring survival (30%) observed even when an attendant male was present. Second, as a direct measure of effect size, these results suggest that the magnitude of the effect of male care on offspring survival between fertilization and hatching may be too small to detect with the sample size we were able to achieve in this field study. From these outcomes, we provisionally conclude that call length is an honest indicator of paternal care quality that females could potentially use during mate selection to gain direct fitness benefits.

Support for the good parent hypothesis has been found previously in both birds with biparental care (Welling et al. 1997; but see Buchanan and Catchpole 2000; Penteriani et al. 2002; Dolby et al. 2005; Hadfield et al. 2006; Bartsch et al. 2015) and fish with male-only care (Knapp and 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), song sequencing (Bartsch et al. 2015), and repertoire size (Buchanan and Catchpole 2000), and characteristics of courtship in fish including courtship rate (Knapp and 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 frogs with parental care, including the golden rocket frog, care for multiple egg clutches simultaneously. Future studies of golden rocket frogs should investigate the extent to which males advertise their paternal care quality directly through the use of male care behaviors.

Our findings do not support the differential allocation hypothesis (Burley 1986), which predicts that attractive males will provide less parental care because either their mates are willing to increase their share so that their offspring will inherit “good genes” or because their mates are willing to compensate for a lack of paternal care. 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, thereby enabling males to decrease their provisioning efforts (Qvarnström 1997; Badyaev and Hill 2002; Johnsen et al. 2005; Maguire and Safran 2010; Limbourg et al. 2013). In the



golden rocket frog, we found a positive correlation between attractiveness (measured in terms of call length) and paternal care quality but no correlation between call length and maternal care quality. While these results do not rule out the possibility that call length may provide some indication of genetic quality, females do not appear to alter their parental care effort according to male attractiveness.

The trade-off hypothesis predicts that when opportunities for matings are abundant, male traits should not necessarily be honest predictors of paternal care quality if males benefit more by increasing mating effort and decreasing parental care effort (Kokko 1998). In golden rocket frogs, multiple egg clutches and tadpoles are often found within the territory of one male (Tumulty 2018), 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 paternal care and male mating effort. We suggest that this absence of a trade-off between mating and parental investments may be a consequence of two features of golden rocket frog breeding and paternal care behaviors. First, male golden rocket frogs are territorial and continue to breed at the sites of current offspring care (Bourne et al. 2001; Pettitt et al. 2012, 2018; Tumulty 2018). As a consequence, the conflicts between mating effort and parental care effort are likely to be minimal or nonexistent (Stiver and Alonzo 2009). Second, the primary form of parental care provided by males—offspring attendance—is “nondepreciable,” that is, 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 primarily in species in which males provide costly, depreciable care (e.g., chick provisioning) that negatively impacts mating effort (Halupka and Borowiec 2006; Mitchell et al. 2007; Diniz et al. 2015).

Finally, we provisionally reject the essential male care hypothesis, which proposes that males reliably signal paternal care quality, that females prefer high-quality males because male care is essential to offspring survival, and that females cannot compensate for low-quality or nonexistent male care (Kelly and Alonzo 2009). Our data indicate that paternal care is not absolutely essential during the embryonic stage of development, because 8% of fertilized eggs with no attending male produced embryos that survived to the tadpole stage after hatching. We suggest that this finding could be due to an increase in parental effort by an attending female, as has been seen in *Allobates femoralis* females (Ringle et al. 2015). On one occasion during our daily surveys, we observed a female transporting a small black tadpole from its natal phytotelm. This represents the first report of a female golden rocket frog transporting young. This tadpole was one of four, out of a clutch size of six, that successfully hatched despite the removal of the attending male. However, the results of our male-removal experiment indicate 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 would remain significant, even if not essential. It also remains to be determined, however, whether paternal care is essential when the entirety of the pre-metamorphic developmental period, which can last more than 6 months (Pettitt 2012), is considered. Hence, our rejection of the essential male care hypothesis remains

provisional until additional data on the tadpole stage of development become available.

### Influence of paternal care on offspring survival

Our results are broadly consistent with findings from 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; Vockenhuber et al. 2009; Seshadri and Bickford 2018). A review by Crump (1995) identified potential functions of parental care in frogs that included reducing risks of predation, desiccation, and fungal infections. In golden rocket frogs, there was a trend for clutches with attendant males present to have lower rates of embryo mortality caused by predation and desiccation compared with clutches for which males were removed. While defensive behavior towards crabs (the most common embryonic predator of golden rocket frogs; Pettitt et al. 2018) was never observed in adults, the presence of attendant males has clear potential to reduce such predation risks. Similar anti-predator benefits attributable to parental care have been identified in other anurans (Juncá 1996; Bourne 1998; Burrows 2000; Bickford 2004; Vockenhuber et al. 2009; Lehtinen et al. 2014). Mortality due to desiccation is one of the most commonly suggested causes of terrestrial anuran offspring loss (Salthe and Mecham 1974; Taigen et al. 1984). Parental behaviors that 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 and Trueb 1994; Bourne 1998). In golden rocket frogs, 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 1983; Bourne 1998), this does not appear to be the case for golden rocket frogs, which showed similar rates of fungal infection in eggs with and without attendant males.

### Female preferences for male calls

Results of our playback tests revealed that female golden rocket frogs preferred longer calls, but they showed no preferences for higher call rates or lower call frequencies. Evidence for directional preferences for longer calls in golden rocket frogs is consistent with the generally held view that females prefer more costly or elaborate male traits (Ryan and Keddy-Hector 1992; Andersson 1994). Based on this same argument, however, we might have also expected to observe a preference for higher call rates. Our demonstrated lack of preference for faster call rates was especially surprising given the results of previous studies looking at the potential role call rate plays in mate selection in this species (Bourne et al. 2001). One potential explanation for this result is based on the presence of a closely related species, Kaie's rocket frog (*Anomaloglossus kaiei*), that breeds sympatrically with golden rocket frogs. This sister species produces a call that is similar to the short, pulsed advertisement call of golden rocket frogs, but it is produced at a faster average call rate (74 calls/min, range: 49–101) compared with golden rocket frogs (26 calls/min, range: 20–41) (Kok et al. 2006; Pettitt et al. 2012; Tumulty 2018). Given that selection should favor signal divergence among closely related sympatric species (Noor 1999), we speculate that a lack of preference for higher call rates in female golden rocket frogs 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; Ryan and Rand 1993; Pfennig 1998, 2000).

Across anuran amphibians, patterns of preference based on differences in call frequency are much more variable than those based on call rate and call duration (Gerhardt and Schwartz 2001; Gerhardt and Huber 2002). Some studies report strong directional preferences (Morris and Yoon 1989; Ryan et al. 1992; Wollerman 1998), while others report stabilizing, weakly directional, or threshold preferences (Grafe 1997; Márquez and Bosch 1997; Schrode et al. 2012; Tanner et al. 2017) or no preference (Lopez and Narins 1991). Even different studies of the same species can produce conflicting results about preferences for call frequency (Morris and Yoon 1989; Schrode et al. 2012; Tanner et al. 2017). Similar to the case for call rate, a lack of preference for lower call frequency in golden rocket frogs may be a result of heterospecific mating avoidance (Höbel and Gerhardt 2003), as the sympatric sister species, Kaiei's rocket frog, produces calls with somewhat lower frequencies (average = 4.85 kHz) compared with golden rocket frogs (average = 5.4 kHz) (Kok et al. 2006; Pettitt et al. 2012; Tumulty 2018). In addition, dominant frequency was not found to be correlated with snout-vent length, mass, or body condition (i.e., size-independent body mass) in male golden rocket frogs (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.

## CONCLUSION

The results of our study are broadly consistent with predictions of the good parent hypothesis, but not with those of the differential allocation, trade-off or essential male care hypotheses. This research is the first to evaluate the predictions of these four hypotheses in a frog with biparental care. The golden rocket frog provides a unique opportunity to test 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 by males versus trophic egg provisioning by females) to offspring at different developmental stages (Bourne et al. 2001; this study). We suggest that, under such conditions, honest signaling of paternal care quality benefits females in the short term and long term. 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 provided during both the egg stage (e.g., attendance) and the lengthy tadpole stage (e.g., tadpole transport). Our study offers the first insights into the role male golden rocket frog advertisement traits play in signaling paternal care quality. Additional evidence is needed to confirm our correlational results and to quantify the fitness benefits received due to female mate selection for these advertisement traits.

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