Co-option of ancestral signal elements in the evolution of a cooperative parental behavior

Authors: Moss, Jeanette B.[1†], Tumulty, James P.[2], and Fischer, Eva K.[1]

Author Affiliations:

[1] Department of Evolution, Ecology, and Behavior, University of Illinois, Urbana-Champaign, Urbana, IL 61801, U.S.A.

[2] Department of Biology, College of William and Mary, Williamsburg, VA, 23185, U.S.A.

[†]Correspondence:

Email: JenBMoss@gmail.com

ABSTRACT

The emergence of new social associations is predicted to be an important selective force in the diversification of communication systems. To meet new functional demands, signal repertoires may expand, or existing signals may be co-opted to solicit distinct, context-dependent responses. In anurans, the vocal repertoires of many species have been characterized in detail, including distinct types of calls for advertisement, courtship, and aggression; yet quantitative descriptions are lacking for social contexts such as parental care. Here, we characterized and compared calls of the biparental poison frog, *Ranitomeya imitator*, across three social contexts: advertisement, courtship, and egg feeding – a unique parenting behavior in which females, coordinated by the calls of their male partners, provision trophic eggs to tadpoles. We found that egg feeding calls shared properties with both advertisement (i.e., longer in duration) and courtship calls (i.e., broader call bandwidths) but were distinct in other properties (i.e., low dominant frequencies and long pulse intervals). Egg feeding calls contained less identity information than advertisement calls but more than courtship calls. Finally, multivariate analysis revealed relatively high classification success for advertisement (82.9%) and courtship calls (73.5%) but misclassified nearly half of egg feeding calls as either advertisement (21.4%) or courtship calls (21.4%). Taken together, egg feeding calls appear intermediate between the two ancestral call types, and likely borrowed and recombined elements of both to solicit a context-dependent parenting response.

Keywords: Acoustic communication, anuran, parental care, tadpole provisioning, social context

INTRODUCTION

Complexity of communication systems is predicted to co-evolve with complexity of social systems (Freeberg *et al.*, 2012; Sewall, 2015; Peckre, Kappeler, & Fichtel 2019). A major reason for this is that transitions in social organization introduce novel contexts for interaction between individuals, which in turn require communication systems that are specialized for more diverse functions. One solution is to increase the size of the signal repertoire through the evolution of entirely novel communication features (Freeberg & Krams, 2015; Broder *et al.*, 2021). Alternatively, existing signals may be co-opted in different contexts to elicit novel behaviors in receivers *via* context-dependent regulation of the response (West-Eberhard, 2003; Rodríguez, 2009). Which pattern prevails – the evolution of novel signal components or context-dependent regulation of the response – depends on whether the costs of producing and processing a novel signal outweigh the costs of an ancestral signal eliciting an inappropriate response in a novel context (Rodríguez, 2009; Broder *et al.*, 2021).

Signals are also composed of multiple components, which may differ in salience to different receivers and be under different forms of selection (Moller & Pomiankowski, 1993; Hebets & Papaj, 2005; Tanner *et al.*, 2017). Therefore, quantitative descriptions of variation in signal components are useful for generating hypotheses about signal function (Gerhardt, 1991; Roberts *et al.*, 2012; Tibbetts *et al.*, 2017; Tumulty *et al.*, 2022). For acoustic signals, Gerhardt, (1991) theorized that signal properties can be placed along a continuum from 'static' to 'dynamic' based on the extent of within-individual variability; static properties often contain information about species identity and are under stabilizing selection while the high variability of dynamic properties may provide information about the quality or condition of the signaler and can be explained by directional selection for extreme values to enhance a signal's motivational

effect (Gerhardt, 1991; Shaw & Herlihy, 2000). Additionally, signal properties that exhibit high levels of variation among individuals but low levels of variation within individuals can provide information about the individual identity of the signaler (Beecher, 1989). In certain social systems, signalers may benefit from being recognized as individuals, and selection can favor greater identity information in signals.

Anurans present a fantastic system for investigating predictions about context specificity and information content of signals in relation to social context. In addition to exhibiting remarkable diversity of mating and social systems, acoustic communication plays a central organizing role in their social behavior (Wells, 1977; Bee *et al.*, 2016). Many species display vocal repertoires of different call types that have different social functions (reviewed in (Wells, 1977, 2010; Toledo *et al.*, 2015); however, evidence for evolutionary lability can be found in both acoustic signals *and* their associated receiver responses. Advertisement calls are the most commonly studied call type for most species (Toledo *et al.*, 2015; Bee *et al.*, 2016); these calls are long-range signals that often serve a dual function of attracting mates and repelling potential rival males (Wells, 1977). Additional call types that are present in some species include courtship calls, which are typically quieter calls that are produced once a female approaches a male, and aggressive calls, which are directed at nearby rival males. In some species the same call, or different components of the same call, may elicit different behaviors in different contexts. For example, the common coquí frog (*Eleutherodactylus coqui*) produces an advertisement call composed of two syllables – 'co' and 'qui' – which are differentially specialized for aggressive versus mate attraction contexts such that regulation of the response is facilitated not only by modification of the syllable composition of calls but also by the evolution of sex-specific auditory sensitivities (Narins & Capranica, 1976). Additionally, *Nyctibatrachus humayuni*

93 produces the same type of call whether signaling to potential mates or territorial rivals, but with
94 clearly distinct behavioral outcomes (Bhat *et al.*, 2022). Clearly, greater complexity of anuran
95 communication systems can evolve through both incorporation of novel signal components as
96 well as co-option of existing signals to new behavioral contexts.

97 One social context in which acoustic communication has not been well studied in frogs is
98 parental care. Some frog species show extensive parental care for eggs and tadpoles, and in cases
99 of biparental care, males and females may need to communicate to coordinate care behaviors.
100 This is the case for mimic poison frogs, *Ranitomeya imitator*, a species characterized by
101 monogamous pair bonding and biparental care of eggs and tadpoles (Brown *et al.*, 2008, 2010).
102 Specifically, females feed tadpoles with unfertilized 'trophic' eggs and males coordinate egg
103 feeding events by calling to females and leading them to hungry, begging tadpoles (Brown *et al.*,
104 2008; Tumulty *et al.*, 2014; Yoshioka *et al.*, 2016). It has been hypothesized that egg feeding
105 behaviors may be evolutionarily derived from courtship behaviors (Caldwell *et al.*, 1999;
106 Poelman & Dicke, 2007; Tumulty *et al.*, 2014). Egg feeding has evolved multiple times in
107 association with transitions to biparental care (Brown *et al.*, 2010) and functions crucially in
108 offspring development (Tumulty *et al.*, 2014), suggesting that selection on signalers and
109 receivers during these interactions is likely strong. However, the vocalizations that coordinate
110 and elicit egg feeding have yet to be quantitatively described for any species, including *R.*
111 *imitator*.

112 Here, we characterize and compare calls of the biparental poison frog, *Ranitomeya*
113 *imitator*, across three social contexts to test the alternative hypotheses that a derived
114 communication function – the coordination of trophic egg feeding within monogamous pairs –
115 has promoted the evolution of new signal components or fine-tuned regulation of the response.

116 Given that several call types in frogs are known to serve dual functions, we predict that egg
117 feeding calls were likely co-opted and modified from calls adapted for the more ancestral
118 functions of advertisement and courtship (described by Mayer *et al.*, 2014 and Twomey *et al.*,
119 2015) and will therefore possess both shared and distinct elements. Further, because egg feeding
120 calls are only likely to be deployed at close range and directed towards a bonded breeding
121 partner, we predicted that the identity information contained in egg feeding calls would be
122 reduced compared to that of advertisement calls.
123
124
125 **MATERIALS AND METHODS**
126 *Animal Husbandry*
127 A captive laboratory colony of *Ranitomeya imitator* descended from five localities
128 (Veradero, Tarapoto, Huallaga, Sauce, Southern, and hybrids between these; Table S1) was
129 maintained in temperature- (71.86 ± 2.79°C) and light- (12:12 h cycle) controlled rooms at the
130 University of Illinois, Urbana-Champaign (UIUC). Male-female breeding pairs were housed in
131 12x12x18 inch glass terraria (©Exo Terra, Mansfield, MA, U.S.A) containing live plants,
132 driftwood, sphagnum moss, leaf litter, and water-filled film cannisters for egg laying and tadpole
133 deposition. Tank humidity (84.95 ± 3.08%) was maintained using a misting system (©Mist King,
134 Ontario, Canada). Frogs were fed wingless *Drosophila* fruit flies dusted with vitamin
135 supplements three times weekly. Only sexually mature individuals were used as focal subjects.
136 All procedures were approved by the UIUC Animal Care and Use committee (Protocol #20147).
137
138 *Acoustic Recordings*

139 Between July 2021 and May 2022, seven male *R. imitator* were recorded in their home
140 terraria across the breeding cycle. A shotgun microphone (K6/ME66, ©Sennheiser,
141 Wennebostel, Germany) was positioned above the screen covering the focal tank and connected
142 to a handheld recorder (H4n Pro, Zoom, Tokyo, Japan); 44.1kHZ sampling rate, 16-bit
143 resolution. For each recording, we documented the time of day and reproductive stage of the
144 focal male by the following categories: (1) nonbreeding (frog did not currently have eggs or
145 tadpoles); (2) brooding (frog had at least one egg but hatching had not yet occurred); or (3)
146 tadpole (at least one tadpole was present in a deposition pool). Across all three stages,
147 advertisement calls were distinguished from shorter courtship calls based on a published
148 characterization of advertisement calls for this species (Twomey, Mayer, and Summers, 2015).
149 While advertisement calls produce a characteristic 'trill' sound and function in long-range mate
150 attraction and territory defense, courtship calls are typically shorter and softer and function in
151 close-range interactions with females (Mayer *et al.*, 2014; Twomey *et al.*, 2015). To isolate
152 vocalizations elicited in the unique social context of egg feeding and test whether these can be
153 quantitatively distinguished from advertisement and courtship calls, we took video recordings
154 (RLC-510A, ©Reolink, New Castle, DE, U.S.A.) concurrently with audio recordings during the
155 tadpole stage. Male *R. imitator* stimulate female partners to lay trophic eggs by leading them to
156 tadpole deposition pools and maintaining close and/or tactile contact while calling continually
157 (Tumulty, Morales, and Summers, 2014). Thus, egg feeding calls were identified as calls
158 coinciding with males moving towards tadpole deposition pools, leading females to pools, sitting
159 on or near the pools, and culminating with the arrival of females at pools. Calls recorded during
160 the tadpole stage while males were away from pools and/or oriented towards egg laying

161 cannisters were identified as either advertisement or courtship calls, as in during nonbreeding
162 and brooding stages.
163
164 *Call Filtering and Analysis*
165 To examine variation within and among the three call types, ten calls of each type for
166 each individual were selected from available recordings. Very low-amplitude or low-quality
167 calls, or calls that could not be clearly classified between the three types on the basis of social
168 context or previous published accounts were excluded. Raw recordings of calls were trimmed in
169 Audacity v. 3.0.2 (Audacity Team, 2021). Analysis of calls was performed in Raven Pro v. 1.6.1
170 (Cornell Lab of Ornithology, 2022). In total, we measured nine acoustic properties of each call.
171 We measured six temporal properties using the entire call or part of the call (i.e., a single pulse
172 or inter-pulse interval) as the unit of measurement in the waveform view in Raven. These
173 included call duration (time in seconds from the first pulse to the last note; Fig. S1A), inter-call
174 interval (time in seconds to the next call within the same bout, with bout defined as calls
175 occurring within one minute of each other; Fig. S1A), number of pulses, pulse duration (time in
176 seconds from the beginning of a note to the end; Fig. S1B), pulse interval (time in seconds from
177 the end of one note to the start of the next note), and pulse rate (the reciprocal of pulse period, or
178 the full time from the beginning of one note to the beginning of next consecutive note; Fig. S1B).
179 Pulse duration, pulse interval, and pulse rate were calculated by taking the average of three notes
180 within the call, excluding introductory notes. Three spectral properties were also quantified using
181 the entire call as the unit of measurement, including the dominant frequency of the call (in Hz;
182 Fig. S1C) and its bandwidth (90%; Fig. S1C). In most cases the dominant frequency
183 corresponded to the second harmonic (5-6 kHz) but occasionally the fundamental frequency (<3

kHz) was dominant and in a few cases, a broad frequency peak in the range of 3-4 kHz was observed and the harmonic structure was unclear (Fig. 1; Fig. S1). Thus, the frequency of the second harmonic was also quantified for each call.

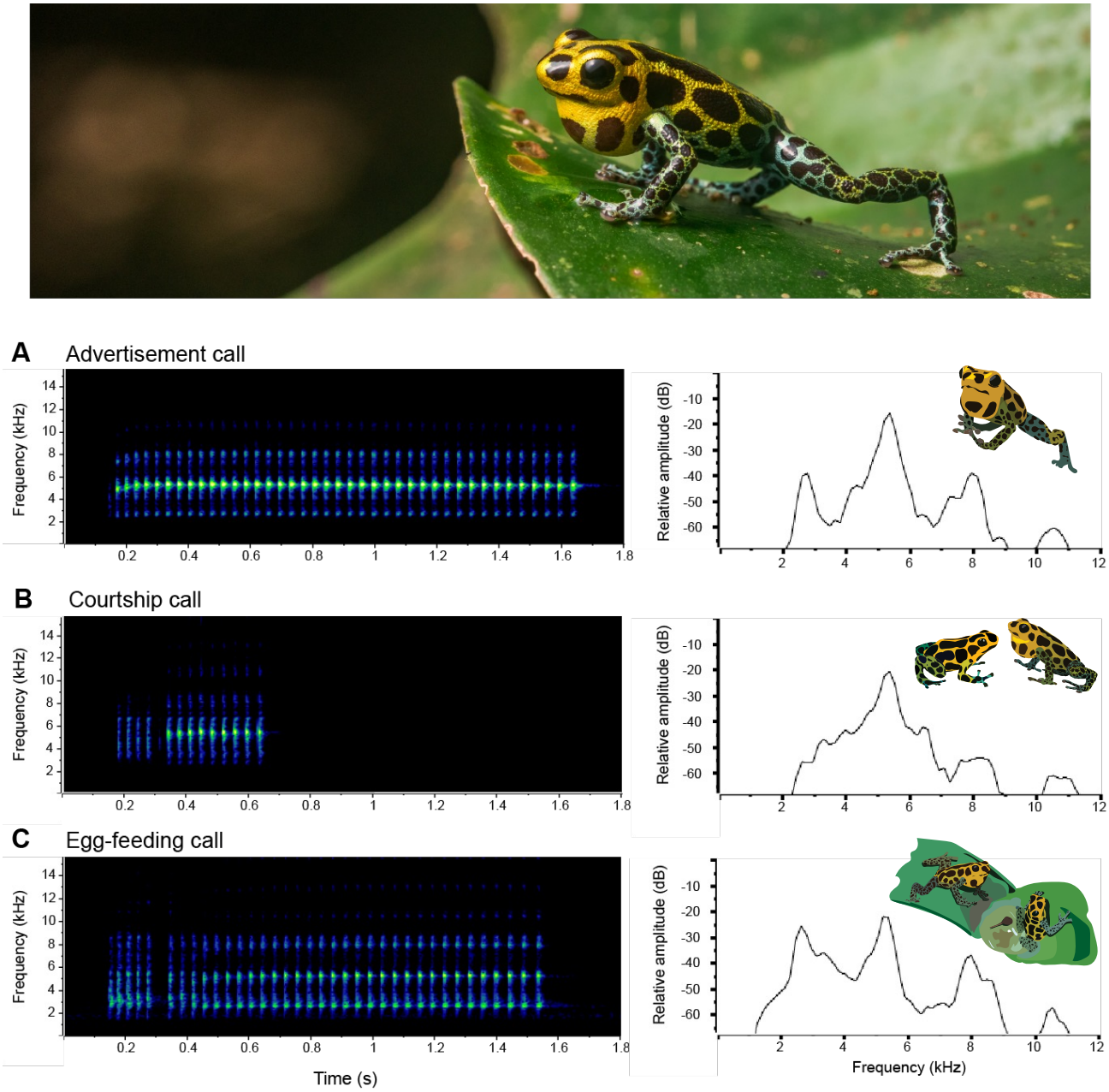

Figure 1: Spectrograms and associated power spectra of a representative (A) advertisement call; (B) courtship call; and (C) egg feeding call of *Ranitomeya imitator* (photo, top). Calls were

visualized in Raven Pro v. 1.6.1 (Ornithology, 2022) using the Hann window, 256 pt frequency resolution. The courtship and egg feeding call power spectra show the two forms of increased frequency bandwidth: broader dominant frequency peak (B) or a fundamental frequency that is at a higher relative amplitude (C). Photo by Anton Sorokin, antonsrkn@gmail.com.

Statistical Analyses

All statistical analyses were performed in R Studio v. 1.1.318 (R Core Development Team, 2020). We first summarized each of nine acoustic properties by their assigned call type. We tested for differences between call types using Type III ANOVAs followed by post-hoc testing. Model testing was carried out using the packages 'lmerTest' (Kuznetsova *et al.*, 2017) and 'emmeans' (Lenth *et al.*, 2022), with reproductive stage specified as a covariate and individual ID specified as a random effect in all models. For metrics that were collected in multiples per call (i.e., pulse duration, pulse interval, and pulse rate), we also specified call ID nested within individual ID as a random effect. Some variables (inter-call interval, pulse interval, pulse rate, and all spectral properties) were log-transformed prior to analysis to better meet assumptions of normality and homogeneity of variance, which we evaluated with analyses of random effects and residuals.

We next evaluated within- and among-individual variation for each call type for each of the nine acoustic properties. For each male, we calculated a within-individual coefficient of variation (CV_w = individual SD/individual mean × 100) and report the mean CV_w across males. Values of CV_w below approximately 5% are considered 'static' call properties, while those above 12% are considered 'dynamic'(Gerhardt, 1991). We also computed the among-individual coefficient of variation (CV_a = grand SD/grand mean × 100) and report the ratio of among-

individual variability (CV_a) to within-individual variability (CV_w) as the potential for individual coding (PIC; (Linhart *et al.*, 2019)). Values of PIC greater than one indicate that a call property varies more among individuals than within individuals. To further describe individual distinctiveness of calls, we calculated Beecher's Information Statistic (H_s; (Beecher, 1989)), which expresses the overall amount of individual identity information contained in calls. To control redundancy due to correlations among measurements, data were transformed into uncorrelated variables using principal components analysis (PCA) and all principal components were retained to calculate H_s (Beecher, 1989). PCAs were implemented with the 'prcomp' function after transforming pooled variables (pulse duration, pulse interval, and pulse rate) into averages to ensure equality of sample sizes and scaling all input variables.

Finally, to describe variation among call types at the population-level and evaluate the success rate of classifying calls to the correct call type (advertisement, courtship, or egg feeding), we used a combination of multivariate and mixed modeling approaches. Data were prepared for multivariate analysis by converting acoustic properties into z-scores scaled to the mean of the individual, which controlled for variation among males. Following standardization, we performed PCA as described above. Principal components with eigenvalues > 1 were extracted as inputs into a discriminant function analysis (DFA). We implemented this test using the linear discriminant analysis ('lda' function) in the package 'MASS' (Venables & Ripley, 2002). Chance-corrected classification success (Cohen's kappa) was calculated following the approach of (Titus *et al.*, 1984) in the R package 'vcd' (Friendly, 2016). Differences in classification success among males were statistically evaluated using a Chi-Squared test of independence.

RESULTS

Acoustic properties vary across three social contexts

All measured acoustic properties except for inter-call interval and pulse rate showed significant variation depending on the associated social context (advertisement, courtship, and egg feeding; Table 1; ANOVA $p < 0.05$). In terms of temporal properties, courtship calls were significantly shorter than advertisement ($t = 6.59$, $p < 0.001$) or egg feeding calls ($t = 4.56$, $p < 0.001$) and contained fewer pulses (advertisement-courtship: $t = 7.24$, $p < 0.001$; courtship-egg feeding: $t = 4.98$, $p < 0.001$). Pulse durations were longer in advertisement calls (advertisement-courtship: $t = 3.06$, $p = 0.003$; advertisement-egg feeding: $t = 4.65$, $p < 0.001$) and shortest in egg feeding calls (egg feeding-courtship: $t = 2.52$, $p = 0.013$; Fig. 2A) while pulse intervals were shorter in advertisement calls (advertisement-egg feeding: $t = 4.22$, $p < 0.001$; advertisement-courtship: $t = 2.72$, $p = 0.007$;) and longest in egg feeding calls (courtship-egg feeding: $t = 2.33$, $p = 0.021$; Fig. 2B). In terms of spectral properties, egg feeding calls had significantly lower dominant frequencies than either advertisement ($t = 3.67$, $p < 0.0001$) or courtship calls ($t = 2.88$, $p = 0.004$; Fig. 2C), and advertisement calls had significantly narrower call bandwidths than courtship ($t = 6.93$, $p < 0.001$) or egg feeding calls ($t = 5.77$, $p < 0.001$; Fig. 2D).

Table 1: Summary of 9 acoustic properties of 70 advertisement calls, 70 courtship calls, and 56 egg feeding calls across seven males. Pulse duration, interval, and rate were measured three times for each call, and summary statistics are thus calculated based on 210 or 168 measurements, respectively. Results of pairwise contrasts performed at the level of each call type are also reported. Tukey-adjusted test statistics (t) and *p*-values are reported for each contrast and significant test-statistics are bolded.

Acoustic Property	**Advertisement**	**Courtship**	**Egg feeding**	**Advertisement-Courtship**		**Advertisement-Egg feeding**		**Courtship-Egg feeding**	
	Mean (± SD)	**Mean (± SD)**	**Mean (± SD)**	**t**	***p***	**t**	***p***	**t**	***p***
Temporal Properties									
Call Duration (s)	1.14 ± 0.23 (0.656–1.734)	0.75 ± 0.29 (0.14–1.92)	1.04 ± 0.35 (0.48–1.97)	**6.589**	**<0.001**	1.072	0.285	**-4.557**	**<0.001**
Call Interval (s)	10.53 ± 7.93 (2.53–40.89)	8.15 ± 5.42 (0.41–34.96)	7.40 ± 5.27 (1.46–28.94)	1.568	0.119	1.424	0.156	0.198	0.843
No. of Pulses	31.33 ± 6.02 (18–46)	20.93 ± 7.28 (5–39)	27.08 ± 9.22 (7–48)	**7.235**	**<0.001**	1.164	0.246	**-4.979**	**<0.001**
Pulse Duration (s)	0.019 ± 0.005 (0.005–0.031)	0.016 ± 0.005 (0.006–0.035)	0.014 ± 0.005 (0.005–0.032)	**3.064**	**0.003**	**4.645**	**<0.001**	**2.519**	**0.013**
Pulse Interval (s)	0.016 ± 0.005 (0.007–0.030)	0.02 ± 0.01 (0.006–0.120)	0.027 ± 0.023 (0.009–0.247)	**-2.722**	**0.007**	**-4.222**	**<0.001**	**-2.330**	**0.021**
Pulse Rate (notes/s)	28.55 ± 2.16 (19.84–37.45)	28.16 ± 3.67 (7.64–37.88)	26.73 ± 5.02 (3.90–38.76)	0.990	0.324	1.907	0.058	1.267	0.207
Spectral Properties									
Dominant Frequency (Hz)	5249.10 ± 496.20 (4048.24–5857.03)	5040.32 ± 612.97 (2842.38–5857.03)	4783.27 ± 779.13 (2625–6201.56)	1.403	0.162	**3.676**	**<0.001**	**2.879**	**0.004**
Frequency of 2nd Harmonic (Hz)	5220.80 ± 601.12 (4048.24–5857.03)	5122.14 ± 495.19 (3789.85–5857.03)	4954.84 ± 542.10 (3617.58–6201.56)	1.520	0.130	**3.946**	**<0.001**	**3.080**	**0.002**
Bandwidth 90% (Hz)	678.83 ± 675.81 (258.40–2928.52)	1544.12 ± 1193.36 (281.25–4823.44)	1662.71 ± 1419.74 (281.25–5426.37)	**-6.932**	**<0.001**	**-5.767**	**<0.001**	-0.413	0.680

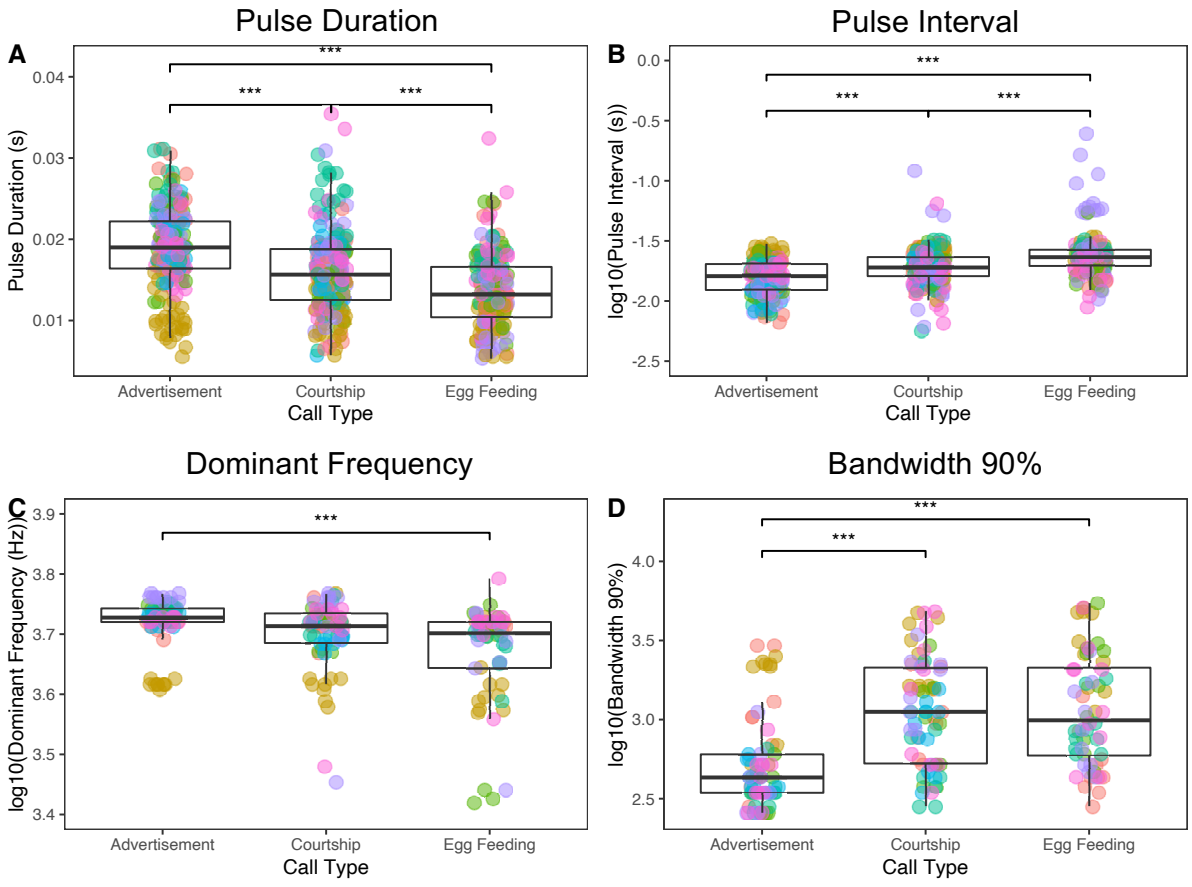

Figure 2: Acoustic properties of *Ranitomeya imitator* vocalizations vary significantly between advertisement, courtship, and egg feeding calls. Plots depict distributions of four acoustic properties by call type: (A) Pulse duration; (B) log10(Pulse interval); (C) log10(Dominant frequency); and (D) log10*Bandwidth 90%). Color of dots corresponds to seven unique males in the sample. Significance levels are shown for Tukey-HSD post-hoc contrasts. Non-significant differences are not shown.

Calls elicited in different social contexts possess different amounts of individual identity information

Putative call types and acoustic properties within those call types also varied in their individual distinctiveness (Table 2). For advertisement calls, dominant frequency and frequency of the 2nd harmonic were classified as 'static' acoustic properties ($CV_w < 5\%$). These same properties for courtship and egg feeding calls, along with pulse rate in advertisement and courtship calls, were classified as 'intermediate' acoustic properties (CV_w between 5% and 12%). All other acoustic properties were classified as 'dynamic ($CV_w > 12\%$). Among calls classified as advertisement calls, most acoustic properties showed greater variation among individuals than within individuals (PIC > 1). The greatest individual distinctiveness was found in spectral properties, in particular dominant frequency (PIC = 5.50). Courtship and egg feeding calls showed less individual distinctiveness overall, with most acoustic properties showing as much or more variation within individuals as among individuals, and dominant frequency being a much less reliable indicator of identity (courtship PIC = 0.63; egg feeding PIC = 0.82). When considering all acoustic properties together, courtship calls contained the least identity information (H_s = 1.48) of the three types (advertisement H_s = 3.82; egg feeding calls H_s = 2.81).

Table 2: Individual distinctiveness of nine acoustic properties of 70 advertisement calls, 70 courtship calls, and 56 egg feeding calls across seven males. Potential for individual coding (PIC) is calculated as the ratio of the variation among individuals to the mean variation within individuals. Values greater than one, indicating that a call property varies more among individuals than within individuals, are shown in bold.

	Advertisement			**Courtship**			**Egg feeding**		
Acoustic Property	**CVa**	**Mean CVw**	**PIC**	**CVa**	**Mean CVw**	**PIC**	**CVa**	**Mean CVw**	**PIC**
Temporal Properties									
Call Duration (s)	11.76	16.74	0.70	13.06	34.36	0.38	20.67	26.24	0.79
Interval to next call (s)	29.56	65.90	0.45	36.30	50.91	0.71	44.70	57.25	0.78

No. of Pulses	**15.39**	**13.43**	**1.15**	18.77	30.32	0.62	**26.43**	**23.62**	**1.12**
Pulse Duration (s)	**20.29**	**19.48**	**1.04**	18.88	26.50	0.71	20.22	27.81	0.73
Pulse Interval (s)	22.21	23.45	0.95	8.43	38.56	0.22	**40.16**	**35.16**	**1.14**
Pulse Rate (notes/s)	5.88	7.59	0.77	5.84	11.02	0.53	9.34	13.95	0.67
Spectral Properties									
Dominant Frequency (Hz)	**9.94**	**1.81**	**5.50**	6.21	10.09	0.63	9.94	12.10	0.82
Frequency of 2nd Harmonic (Hz)	**11.35**	**3.81**	**2.98**	**7.37**	**6.49**	**1.14**	**9.6**	**6.58**	**1.46**
Bandwidth 90% (Hz)	**70.83**	**47.50**	**1.49**	48.57	62.18	0.78	58.74	62.49	0.94

High classification success of vocalizations when controlling for individual variation

Principal components analysis (PCA) of all call types across all individuals returned three primary axes of variation (eigenvalues > 1) which together explained 67.8% of variation in acoustic properties (Table S2). Pulse duration, pulse interval, dominant frequency, and frequency of the 2nd harmonic all loaded heavily on the first principal component (PC1), while call duration and number of pulses loaded heavily on the second principal component (PC2; Table S2). The third principal component (PC3) contributed only subtly to variation among call types, with variation explained by call interval and pulse duration. Overlap between call types was evident across all individuals assayed (Fig. S2–3).

Using PCs 1–3 as inputs, discriminant function analysis (DFA) correctly classified 72.2% of calls to their assigned call type. The chance-corrected classification success was significant (Weighted Cohen's kappa = 0.566, $p < 0.001$). The strongest coefficients of the first linear discriminant were held by PC1 (the 'frequency and pulse period' factor), whereas PC2 (the 'call length' factor) contributed more to the second linear discriminant for distinguishing call types. The highest accuracy of classification according to this model was for advertisement calls (82.9%) followed by courtship calls (73.5%) and finally egg feeding calls (57.1%). Courtship calls were equally likely to be misclassified as advertisement (13.2%) or egg feeding calls

(13.2%), and egg feeding calls were equally likely to be misclassified as advertisement (21.4%) or courtship calls (21.4%). While overall misclassification rates did not vary significantly across males (27.7 ± 9.31%; $\chi^2 = 21$, $p = 0.279$), over half of all misclassified egg feeding calls (16/24) were attributed to three individuals: Ri.0181, Ri.0187, and Ri.0442 (Fig S3).

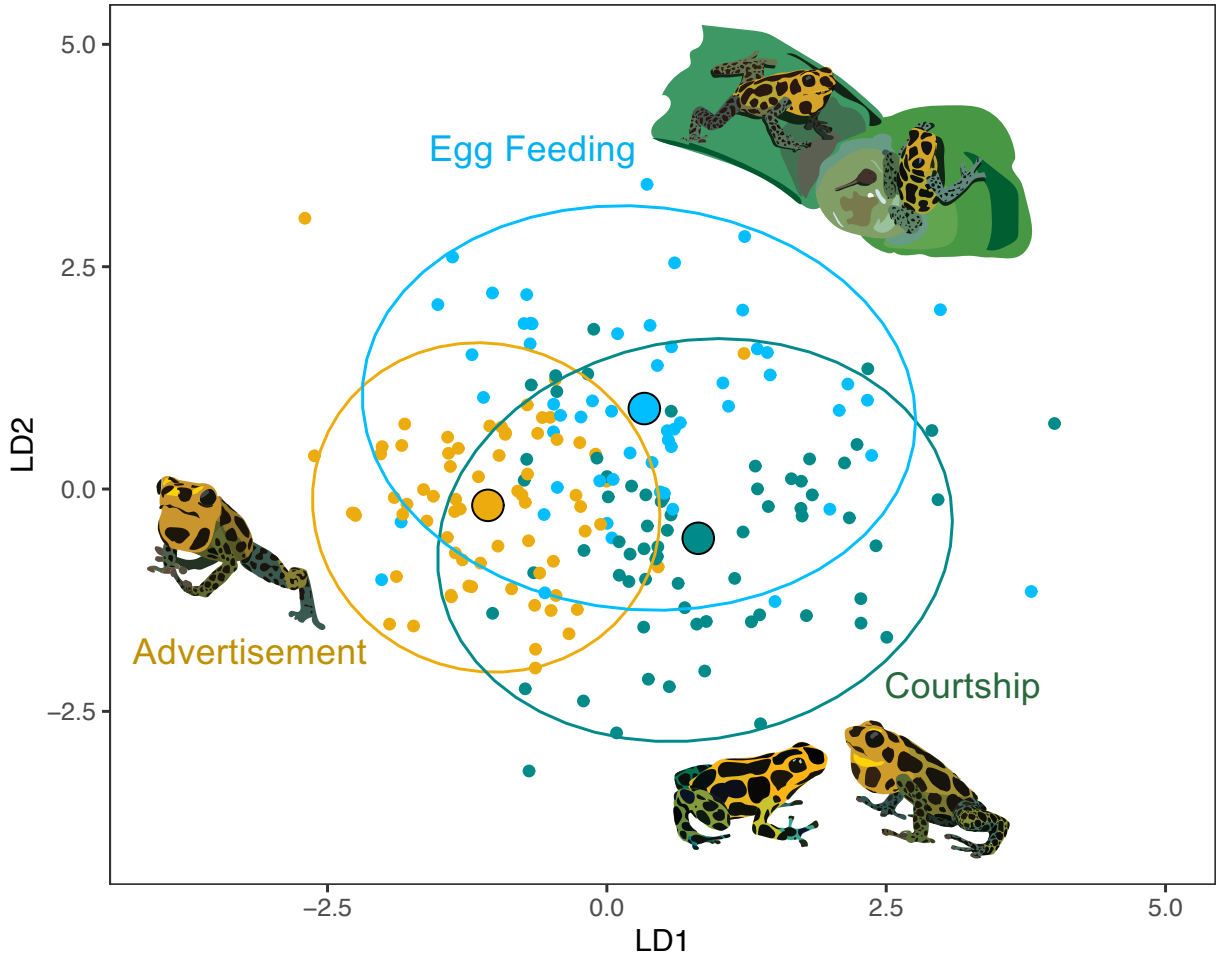

Figure 3: Plot of 196 *Ranitomeya imitator* calls in two-dimensional space defined by the first two discriminant functions of a linear discriminant function analysis (DFA). Calls are clustered by call type (advertisement, courtship, and egg feeding) with centroids (large points) and 95% confidence intervals (ellipses) depicted for each type.

DISCUSSION

Increased complexity of communication systems is predicted to co-evolve with the emergence of new forms of social interaction (Freeberg *et al.*, 2012; Freeberg & Krams, 2015; Sewall, 2015; Peckre, Kappeler, & Fichtel 2019). Such complexity can arise *via* evolutionary mechanisms affecting the signaler, the receiver, or both, depending on how selection acts on novel versus ancestral signals when expressed in a new context (Rodríguez, 2009; Broder *et al.*, 2021). In this study, we characterized and compared calls of the biparental poison frog, *Ranitomeya imitator*, across social contexts in which calls either serve an ancestral communication function – advertisement or courtship – or a derived social function – egg feeding. We found that vocalizations differ in both acoustic properties and the amount of identity information contained in those properties across ancestral and derived social contexts. Crucially, calls elicited during egg feeding possessed unique spectral and temporal properties that were not observed in advertisement or courtship contexts and retained moderate identity information. Despite this, nearly half of all egg feeding calls were misclassified on the basis of acoustic properties alone, suggesting that egg feeding calls were likely co-opted from existing elements but have since undergone evolutionary modification in form.

Studies of anuran acoustic communication recognize multiple common 'types' of calls that function in distinct social contexts (Toledo *et al.*, 2015). Advertisement calls are by far the most studied anuran call type because they serve multiple social functions (i.e., mate attraction, territory defense, and species delimitation) and are therefore taxonomically widespread (Toledo *et al.*, 2015; Bee *et al.*, 2016). Comparatively less attention has been paid to characterizing vocalizations that have evolved for other social contexts such as biparental care, in which communication between the sexes holds a special significance beyond simple mate attraction.

342 We quantified nine acoustic properties of vocalizations elicited during egg feeding in *R. imitator*
343 and compared these to the same properties quantified for advertisement and courtship calls. We
344 found that egg feeding calls shared some properties with advertisement calls (i.e., longer than
345 courtship calls with more pulses) and some with courtship calls (i.e., broader call bandwidths),
346 supporting an evolutionary co-option scenario. This is not surprising, as male advertisement and
347 courtship calls already function to elicit a female response and therefore may not be as
348 immediately costly to process in a new social context as novel signal elements. However, we
349 also identified properties in egg feeding calls that were distinct from either ancestral call type
350 (i.e., low dominant frequencies and especially short pulse durations coupled with long pulse
351 intervals). Some of these differences in acoustic properties could be physiologically related to
352 egg feeding calls being produced at lower amplitudes (i.e., "quieter") because pairs are typically
353 very close in space (<0.5 m) when males are leading females to tadpoles and stimulating them to
354 feed. However, courtship calls are also "quiet" close-range signals, so the unique elements of egg
355 feeding calls cannot be entirely explained as physiological by-products of low amplitude calls.
356 Modification of temporal patterning appears to be a common theme in the evolution of context-
357 dependent signals (e.g., Gentner *et al.*, 2006; Bhat *et al.*, 2022) and thus such subtle differences
358 could reflect novel elements that are selectively maintained for the coordination of egg feeding.
359 A second major finding of our study was that the individual distinctiveness of *R. imitator*
360 vocalizations varies according to the social context in which they are deployed. By measuring
361 calls repeatedly and across multiple social behaviors in the same seven males, we showed that
362 advertisement calls contain the most individual identity information overall, with dominant
363 frequency being the most reliable signature of male caller. Dominant frequency is relatively
364 'static' within males of many species (e.g., Gerhardt, 1991; Bee & Gerhardt, 2001) presumably

365 because it is constrained by the morphology of the sound producing structures (Martin, 1971)
366 and thus correlated with body size. Individually distinctive advertisement calls may be a signal
367 or cue of individual identity used by rival males to recognize territory neighbors (Bee &
368 Gerhardt, 2002; Chuang *et al.*, 2017; Tumulty & Bee, 2021). In the case of mimic poison frogs,
369 which are characterized by monogamous pair bonds, individually distinctive advertisement calls
370 could also be used by females to recognize their mate. If males benefit from being recognized by
371 either territory neighbors or mates, selection could favor greater identity information in these
372 calls to facilitate recognition. A complementary explanation is that courtship and egg feeding
373 calls are typically produced over shorter distances than advertisement calls, such that other signal
374 modalities (e.g., visual and chemosensory cues) may take the place of auditory cues in mediating
375 individual recognition. Indeed, the majority of acoustic properties examined were more dynamic
376 (i.e., showed more within-individual variation) in courtship and egg feeding calls than in
377 advertisement calls, which could reflect stronger selection for extreme values to enhance the
378 motivational effects of these signals.

379 Finally, we evaluated whether subtle variation in the spectral range, temporal patterning,
380 and individual distinctiveness of egg feeding calls was sufficient to quantitatively distinguish
381 them from ancestral call types. In a discriminant function analysis, all three call types were
382 classified above chance level (33%) from their acoustic properties alone; however, overlap
383 between call types was considerable (Fig. 3A) and calls elicited during egg feeding were
384 misclassified in nearly half of all instances. Thus, the argument can be made that egg feeding
385 calls are not distinct enough to afford their own category within the vocal repertoire of *R.*
386 *imitator*. An intriguing observation can be made, however, in the patterns of misclassification.
387 Egg feeding calls in our sample were equally likely to be misclassified as advertisement or

388 courtship calls, suggesting that egg feeding calls borrow and recombine elements from multiple
389 call types. Perhaps this complexity arises from the unique function of egg feeding calls, which
390 may align selection on the response with both advertisement (i.e., must attract females to a
391 specific location) and courtship calls (i.e., must motivate females to lay eggs). Additionally,
392 females likely gather other contextual cues and signals when deciding to lay trophic eggs (which
393 are typically laid in the water) versus eggs that will be fertilized (which are typically attached to
394 a leaf above the water line or in a location away from a pool). For example, tadpoles of this
395 species vibrate vigorously against parental frogs to signal their hunger, and this additional signal
396 appears to be important in eliciting trophic eggs (Yoshioka *et al.*, 2016). Future studies should
397 focus on female perception of male call variants in relation to other contextual cues to better
398 understand how selection on signalers and receivers could synergistically drive the co-option and
399 modification of acoustic signals for trophic egg feeding.

400 In conclusion, our study examined acoustic signals produced in a derived social context
401 in biparental poison frogs and contrasted these against ancestral signal types adapted to function
402 in other facets of male-female communication. We found support for the prediction that extended
403 functionality of the communication system in association with increased social complexity likely
404 arose through co-option of multiple pre-existing signals followed by evolutionary modification,
405 resulting in novel elements. Our results highlight the complexity of anuran communication
406 systems and the need to characterize vocal repertoires across a greater diversity of social
407 contexts. Furthermore, this study contributes to understanding how signals are evolutionarily
408 fine-tuned to convey context-dependent information to receivers.

409

410

REFERENCES

Bee, M.A. & Gerhardt, H.C. 2002. Individual voice recognition in a territorial frog (*Rana catesbeiana*). *Proc. R. Soc. Lond. B Biol. Sci.* **269**: 1443–1448.

Bee, M.A. & Gerhardt, H.C. 2001. Neighbour–stranger discrimination by territorial male bullfrogs (*Rana catesbeiana*): I. Acoustic basis. *Anim. Behav.* **62**: 1129–1140.

Bee, M.A., Reichert, M.S. & Tumulty, J. 2016. Assessment and recognition of rivals in anuran contests. In: *Advances in the Study of Behavior*, pp. 161–249. Elsevier.

Beecher, M.D. 1989. Signalling systems for individual recognition: an information theory approach. *Anim. Behav.* **38**: 248–261. Academic Press.

Bhat, A.S., Sane, V.A., Seshadri, K.S. & Krishnan, A. 2022. Behavioural context shapes vocal sequences in two anuran species with different repertoire sizes. *Anim. Behav.* **184**: 111–129.

Broder, E.D., Elias, D.O., Rodríguez, R.L., Rosenthal, G.G., Seymoure, B.M. & Tinghitella, R.M. 2021. Evolutionary novelty in communication between the sexes. *Biol. Lett.* **16**: 20200733.

Brown, J.L., Morales, V. & Summers, K. 2010. A key ecological trait drove the evolution of biparental care and monogamy in an amphibian. *Am. Nat.* **175**: 436–446.

Brown, J.L., Twomey, E., Morales, V. & Summers, K. 2008. Phytotelm size in relation to parental care and mating strategies in two species of Peruvian poison frogs. *Behaviour* **145**: 1139–1165.

Caldwell, J.P., de Oliveira, V.R.L. & de Oliveira, V.R.L. 1999. Determinants of biparental care in the spotted poison frog, *Dendrobates vanzolinii* (Anura: Dendrobatidae). *Copeia* **1999**: 565.

Chuang, M.-F., Kam, Y.-C. & Bee, M.A. 2017. Territorial olive frogs display lower aggression towards neighbours than strangers based on individual vocal signatures. *Anim. Behav.* **123**: 217–228.

Cornell Lab of Ornithology. 2022. Raven Pro: Interactive Sound Analysis Software (Version 1.6.3). The Cornell Lab of Ornithology, Ithaca, NY, U.S.A.

Freeberg, T.M., Dunbar, R.I.M. & Ord, T.J. 2012. Social complexity as a proximate and ultimate factor in communicative complexity. *Philos. Trans. R. Soc. B Biol. Sci.* **367**: 1785–1801. The Royal Society.

Freeberg, T.M. & Krams, I. 2015. Does social complexity link vocal complexity and cooperation? *J. Ornithol.* **156**: 125–132.

Friendly, M. 2016. Working with categorical data with R and the vcd and vcdExtra packages.

Gentner, T.Q., Fenn, K.M., Margoliash, D. & Nusbaum, H.C. 2006. Recursive syntactic pattern learning by songbirds. *Nature* **440**: 1204–1207.

Gerhardt, H.C. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. *Anim. Behav.* **42**: 615–635. Academic Press.

Hebets, E.A. & Papaj, D.R. 2005. Complex signal function: developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* **57**: 197–214.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* **82**: 1–26. American Statistical Association.

Lenth, R.V., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H., *et al.* 2022. Package "emmeans" Type Package Title Estimated Marginal Means, aka Least-Squares Means. *R Package Version 340*, doi: 10.1080/00031305.1980.10483031.

Linhart, P., Osiejuk, T.S., Budka, M., Šálek, M., Špinka, M., Policht, R., *et al.* 2019. Measuring individual identity information in animal signals: Overview and performance of available identity metrics. *Methods Ecol. Evol.* **10**: 1558–1570. John Wiley & Sons, Ltd.

Martin, W.F. 1971. Mechanics of sound production in toads of the genus *Bufo*: Passive elements. *J. Exp. Zool.* **176**: 273–293.

Mayer, M., Schulte, L.M., Twomey, E. & Lötters, S. 2014. Do male poison frogs respond to modified calls of a Müllerian mimic? *Anim. Behav.* **89**: 45–51.

Moller, A.P. & Pomiankowski, A. 1993. Why have birds got multiple sexual ornaments? *Behav. Ecol. Sociobiol.* **32**.

Narins, P.M. & Capranica, R.R. 1976. Sexual differences in the auditory system of the tree frog *Eleutherodactylus coqui*. *Science* **192**: 378–380.

Peckre, L., Kappeler, P.M., & Fichtel, C. 2019. Clarifying and expanding the social complexity hypothesis for communicative complexity. *Behav. Ecol. Sociobiol.* **73**: 11.

Poelman, E.H. & Dicke, M. 2007. Offering offspring as food to cannibals: oviposition strategies of Amazonian poison frogs (*Dendrobates ventrimaculatus*). *Evol. Ecol.* **21**: 215–227.

R Core Development Team. 2020. R: A language and environment for statistical computing.

Roberts, A.I., Vick, S.-J., Roberts, S.G.B., Buchanan-Smith, H.M. & Zuberbühler, K. 2012. A structure-based repertoire of manual gestures in wild chimpanzees: Statistical analyses of a graded communication system. *Evol. Hum. Behav.* **33**: 578–589.

Rodríguez, R.L. 2009. Trait duplication by means of sensory bias. *Behav. Ecol.* **20**: 1376–1381. Oxford Academic.

Sewall, K.B. 2015. Social complexity as a driver of communication and cognition. *Integr. Comp. Biol.* **55**: 384–395. Oxford Academic.

Shaw, K.L. & Herlihy, D.P. 2000. Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. *Proc. R. Soc. Lond. B Biol. Sci.* **267**: 577–584.

Tanner, J.C., Ward, J.L., Shaw, R.G. & Bee, M.A. 2017. Multivariate phenotypic selection on a complex sexual signal. *Evolution* **71**: 1742–1754.

Tibbetts, E.A., Mullen, S.P. & Dale, J. 2017. Signal function drives phenotypic and genetic diversity: The effects of signaling individual identity, quality or behavioural strategy. *Philos. Trans. R. Soc. B Biol. Sci.* **372**: 20160347.

Titus, K., Mosher, J.A. & Williams, B.K. 1984. Chance-corrected classification for use in discriminant analysis: ecological applications. *Am. Midl. Nat.* **111**: 1–7.

Toledo, L.F., Martins, I.A., Bruschi, D.P., Passos, M.A., Alexandre, C. & Haddad, C.F.B. 2015. The anuran calling repertoire in the light of social context. *Acta Ethologica* **18**: 87–99.

Tumulty, J., Morales, V. & Summers, K. 2014. The biparental care hypothesis for the evolution of monogamy: Experimental evidence in an amphibian. *Behav. Ecol.* **25**: 262–270.

Tumulty, J.P. & Bee, M.A. 2021. Ecological and social drivers of neighbor recognition and the dear enemy effect in a poison frog. *Behav. Ecol.* **32**: 138–150.

Tumulty, J.P., Fouilloux, C.A., Goyes Vallejos, J. & Bee, M.A. 2022. Predicting and measuring decision rules for social recognition in a neotropical frog. *Am. Nat.* **200**: E77–E92.

Twomey, E., Mayer, M. & Summers, K. 2015. Intraspecific call variation in the mimic poison frog *Ranitomeya imitator*. *Herpetologica* **71**: 252–259.

Venables, W.N. & Ripley, B.D. 2002. *Modern Applied Statistics with S-PLUS*, Fourth. Springer, New York, NY, USA.

Wells, K.D. 2010. *The Ecology and Behavior of Amphibians*. University of Chicago Press, Chicago.

Wells, K.D. 1977. The social behaviour of anuran amphibians. *Anim. Behav.* **25**: 666–693.

West-Eberhard, M.J. 2003. *Developmental plasticity and evolution*. Oxford University Press, Oxford, U.K.

Yoshioka, M., Meeks, C. & Summers, K. 2016. Evidence for begging as an honest signal of offspring need in the biparental mimic poison frog. *Anim. Behav.* **113**: 1–11.