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Evolution of acoustic signals associated with cooperative parental behavior in a poison frog

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The emergence of complex social interactions is predicted to be an important selective force in the diversification of communication systems. Parental care presents a key social context in which to study the evolution of novel signals, as care often requires communication and behavioral coordination between parents and is an evolutionary stepping-stone toward increasingly complex social systems. Anuran amphibians (frogs and toads) are a classic model of acoustic communication and the vocal repertoires of many species have been characterized in the contexts of advertisement, courtship, and aggression, yet quantitative descriptions of calls elicited in the context of parental care are lacking. The biparental poison frog, *Ranitomeya imitator*, exhibits a remarkable parenting behavior in which females, cued by the calls of their male partners, feed tadpoles unfertilized eggs. Here, we characterized and compared calls across three social contexts, for the first time including a parental care context. We found that egg-feeding calls share some properties with both advertisement and courtship calls but also had unique properties. Multivariate analysis revealed high classification success for advertisement and courtship calls but misclassified nearly half of egg feeding calls as either advertisement or courtship calls. Egg feeding and courtship calls both contained less identity information than advertisement calls, as expected for signals used in close-range communication where uncertainty about identity is low and additional signal modalities may be used. Taken together, egg-feeding calls likely borrowed and recombined elements of both ancestral call types to solicit a novel, context-dependent parenting response.

animal communication | anuran | parental care | cooperation | social context

Complexity of communication systems is predicted to coevolve with complexity of social systems (1, 2). Transitions in social organization introduce novel contexts for interaction between individuals, which in turn require more specialized communication systems. Such links have been reported across diverse taxa (i.e., birds, mammals, reptiles, amphibians, and insects) and modes of communication (3). While traditionally examined through the lens of group size (4–6), there is increasing interest in understanding how these coevolutionary dynamics play out across multiple indices of social complexity (3, 7, 8). Parental care is a major component of a species' social system (9) and represents a key step in the evolution of complex sociality (10). When care responsibilities are shared between multiple caregivers, ensuring offspring survival requires coordinated behaviors that rely on effective communication. One solution is to expand the size of the signal repertoire by adding novel, context-specific communication features (7, 11), as has been shown in the case of avian cooperative breeding (8). Alternatively, existing signals may be co-opted to function across social contexts via context-dependent regulation of responses (12, 13), as with the versatile alarm calls of some monkeys (14, 15). Quantitative descriptions of variation in signal components provide an important first step for generating hypotheses about signal function and evolution.

Anuran amphibian vocal communication and auditory processing is one of the most influential models for studying animal communication. Across frogs and toads, vocal repertoires of differing sizes and compositions have been meticulously characterized along with the diversity of behavioral and neural responses they elicit (16–18). This rich literature illustrates how greater complexity of communication systems may evolve through the specialization of signal components for a given behavioral context, evolution of receiver bias, or both. In a classic example, the common coquí frog (*Eleutherodactylus coqui*), advertisement call is composed of two syllables—"co" and "qui"—which are differentially specialized for aggressive versus mate attraction contexts (19). In this case, regulation of responses is further facilitated by the evolution of sex-specific auditory sensitivities (19). Conversely, modulation of the túngara frog's (*Physalaemus pustulosus*) characteristic "whine-chuck" call has similar motivating effects on potential mates and rivals, but with clearly distinct social and behavioral outcomes (20).

Significance

Parental care has evolved independently in every major animal lineage and represents a key step in the evolution of complex sociality. When parental care requires coordination between parents, communication systems may increase in complexity alongside parental behavior. To explore this possibility, we characterized calls associated with trophic egg feeding, a unique cooperative parental behavior, in a biparental poison frog. We tested the idea that egg feeding calls are distinct from calls produced during mate attraction. Our analysis revealed considerable overlap between call types, but with some unique properties, suggesting that signals deployed in a novel social context evolve via recombination and modification of existing signals. These findings deepen our understanding of the relationship between complexity of social and communication systems.

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Beyond serving as models for the study of animal communication, anurans also exhibit remarkable diversity of reproductive modes and parental care (18, 21, 22). Indeed, parental care has independently evolved and diversified more frequently among frogs than any other tetrapod group (reviewed in ref. 21). The mimic poison frog, *Ranitomeya imitator*, is a species characterized by monogamy, pair bonding, and biparental care of eggs and tadpoles (23). Terrestrial eggs are cared for by parents and tadpoles are transported "piggy-back," typically by the male, to pools of water upon hatching. Parents then visit and care for their developing tadpoles, with females feeding tadpoles unfertilized "trophic" eggs until metamorphosis. This fascinating behavior is coordinated by males calling to females and leading them to hungry, begging tadpoles (24). While male stimulation is not essential for egg feeding, tadpole growth and survival is significantly improved by male involvement during this stage (25). Egg feeding has evolved multiple times in association with biparental care and the use of small, resource limited pools for breeding (23) and represents a highly derived trait in Dendrobatids (Fig. 1). Because coordination between parents is crucial to offspring development (24), selection on signalers (males) and receivers (females) during these interactions must be strong. It has been hypothesized that egg-feeding behaviors are evolutionarily derived from courtship behaviors (25–27); however, the signals that coordinate and elicit egg feeding have yet to be quantitatively described for any species, including *R. imitator*.

Here, we characterize and compare calls of *R. imitator* across ancestral mating and novel parental contexts to test whether the emergence of coordinated parental care has promoted acoustic signal evolution in this lineage. To reliably elicit a novel parental behavior, we predicted that egg-feeding calls would be acoustically distinct from advertisement and courtship calls (described in refs. 29 and 30), although we anticipated some properties would overlap due to morphological constraints of call production. An alternative is that ancestral signals were co-opted in their original form to function in the novel parental care context. Either scenario sheds light into how systems of communication co-evolve with complexity of parental care.

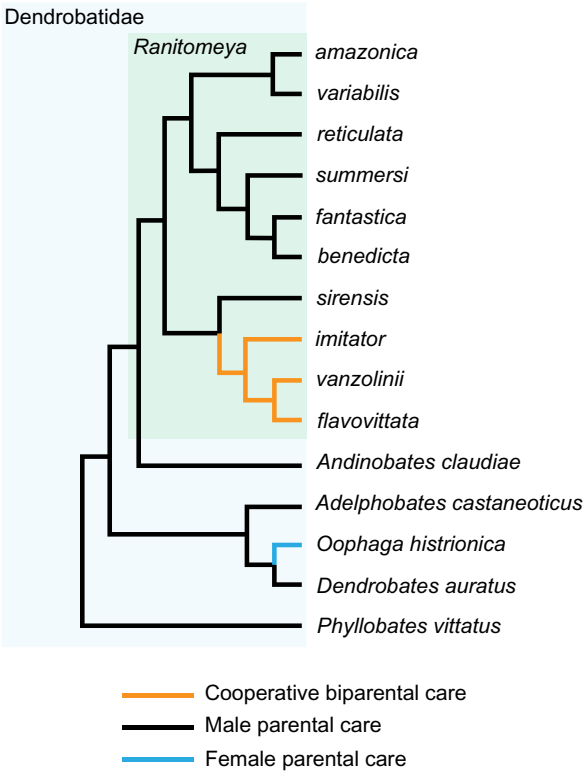

Fig. 1. Phylogeny, redrawn and trimmed from Brown et al. (28), illustrating the diversification of parental care behaviors in *Ranitomeya* and other representative species of Dendrobatidae (note: branch lengths are not meaningful and only a subset of representative species outside of *Ranitomeya* are shown). The ancestral state of this family is male parental care; cooperative biparental care with egg feeding (gold branches) is a highly derived state occurring only in the *Ranitomeya vanzolinii* group. References for character states are provided in Dataset S1.

Results

Calls Vary across Social Contexts. To quantify and compare advertisement, courtship, and egg feeding signals, we recorded representative calls of each type for captive male *R. imitator*. Briefly, advertisement calls—long-range signals that function in mate attraction and territory defense—and courtship calls—short-range signals used in mate attraction (16, 17) were distinguished based on published descriptions of *R. imitator* vocalizations (29, 30), whereas egg feeding calls were identified using video surveillance of parenting frogs (e.g., Movie S1). The visual inspection of waveforms and spectrograms of putative call types revealed distinct characteristics (Fig. 2), although not all features were present across all calls. For example, only some calls contained low-amplitude introductory pulses (*SI Appendix*, Fig. S1).

Because signals are composed of multiple components, which may differ in salience to different receivers and be under different forms of selection (32, 33), we measured nine acoustic properties to quantify call variation (*SI Appendix*, Table S2 and Fig. S2). All properties except for inter-call interval and pulse rate showed significant variation depending on the social context in which the call was measured (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 ($t = 4.56$, $P < 0.001$) (*SI Appendix*, Fig. S3*A*) and contained fewer pulses (advertisement-courtship: $t = 7.24$, $P < 0.001$; courtship-egg feeding: $t = 4.98$, $P < 0.001$) (*SI Appendix*, Fig. S3*C*). Pulse durations were longest 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. 3*A*), while pulse intervals were shortest 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. 3*B*). In terms of spectral properties, the dominant frequency (typically the second harmonic) was significantly lower in egg feeding calls compared to advertisement ($t = 3.67$, $P < 0.0001$) and courtship calls ($t = 2.88$, $P = 0.004$;) (Fig. 3*C*). These differences in spectral properties reflect, at least in part, differences in the distribution of sound energy across a call's frequencies. For example, the fundamental frequency was at a relatively higher amplitude in many courtship and egg-feeding calls, and was occasionally the dominant frequency (Fig. 2*C*). Further, the call bandwidth (i.e., the range in frequency across 90% of a call's sound energy, *SI Appendix*, Fig. S2*C*), was significantly narrower for advertisement calls than for courtship ($t = 6.93$, $P < 0.001$) or egg feeding calls ($t = 5.77$, $P < 0.001$) (Fig. 3*D*).

Individual Identity Information Varies across Call Types. We next examined the degree to which acoustic properties of each call type conveyed information about signaler identity. We found that putative call types and acoustic properties within those call types varied in their individual distinctiveness (Table 1). A key criterion for conveying information about signaler identity is that signal properties exhibit high levels of variation among individuals

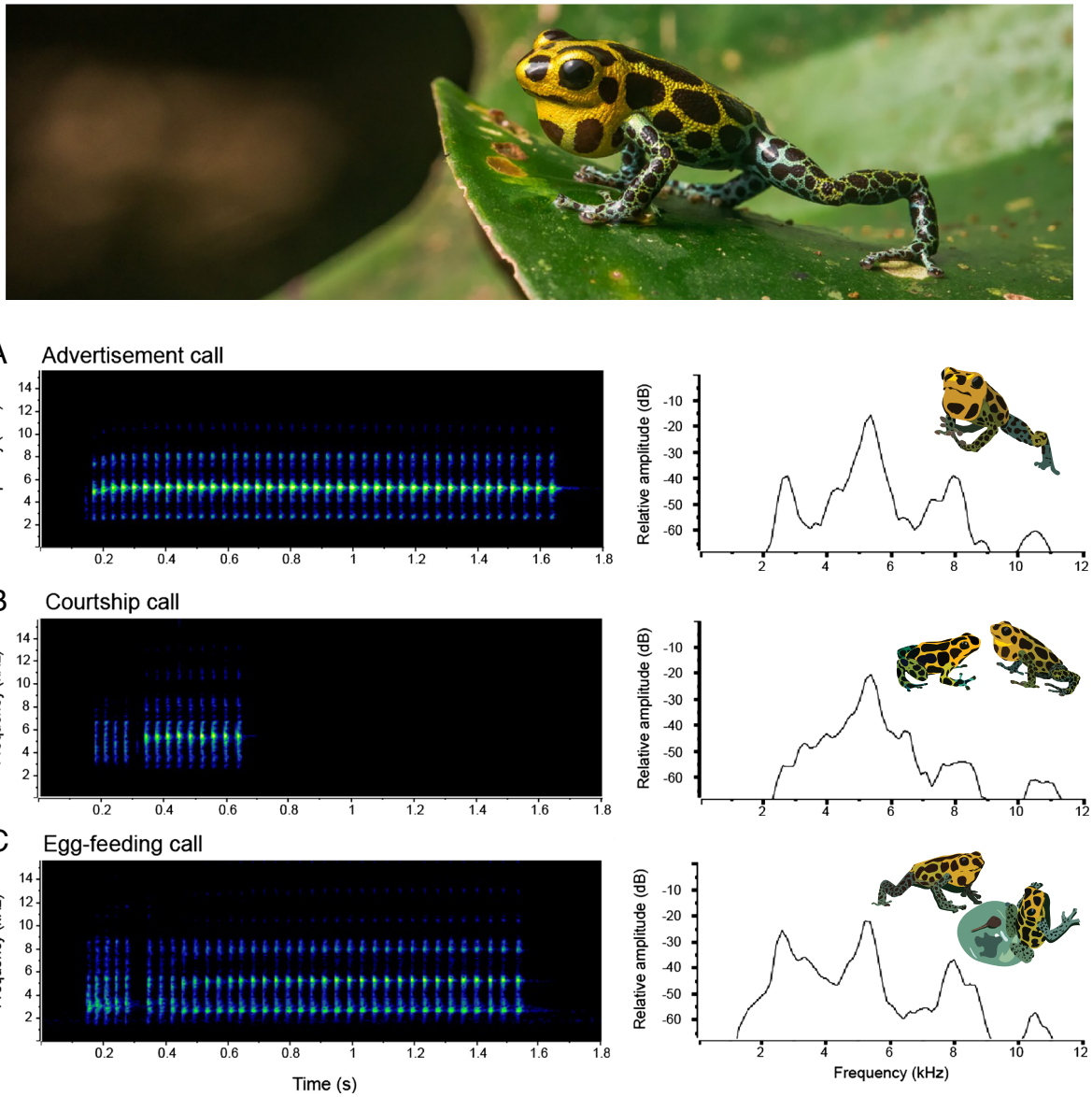

Fig. 2. Vocalizations have unique spectral profiles across social contexts. Spectrograms and associated power spectra of representative (*A*) advertisement call; (*B*) courtship call; and (*C*) egg feeding call of *R. imitator* (photo, *Top*). Calls were visualized in Raven Pro v. 1.6.1 (31) using the 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*). See *SI Appendix*, Fig. S1 for variations on these patterns. Photo by Anton Sorokin, antonsrkn@gmail.com.

(CV_a) but low levels of variation within individuals (CV_w; (34). For calls classified as advertisement calls, most acoustic properties showed greater variation among individuals than within individuals (CV_a/CV_w, or potential for individual coding (PIC; (35)) > 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 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).

High Call Type Classification Success. To describe variation among call types at the population level, we performed a principal components analysis (PCA). This returned three primary axes of variation (eigenvalues > 1) which together explained 67.8% of variation in acoustic properties (*SI Appendix*, Table S3). Pulse duration, pulse interval, dominant frequency, and frequency of the second harmonic all loaded heavily on the first principal component (PC1; 37.8%), while call duration and number of pulses loaded heavily on the second principal component (PC2; 18.2%) (*SI Appendix*, Table S3). The third principal component (PC3; 11.8%) 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 (*SI Appendix*, Figs. S4 and S5), demonstrating that population-level patterns were not obscured by variation at the individual level.

To evaluate the predictive capacity of the PCs in assigning calls to their predefined social contexts, we next performed a discriminant function analysis (DFA) using PCs 1–3 as inputs. This approach correctly classified 72.2% of calls to their assigned social context. The chance-corrected classification success was significant (chance level = 33%; Weighted Cohen's kappa = 0.566, $P < 0.001$). The

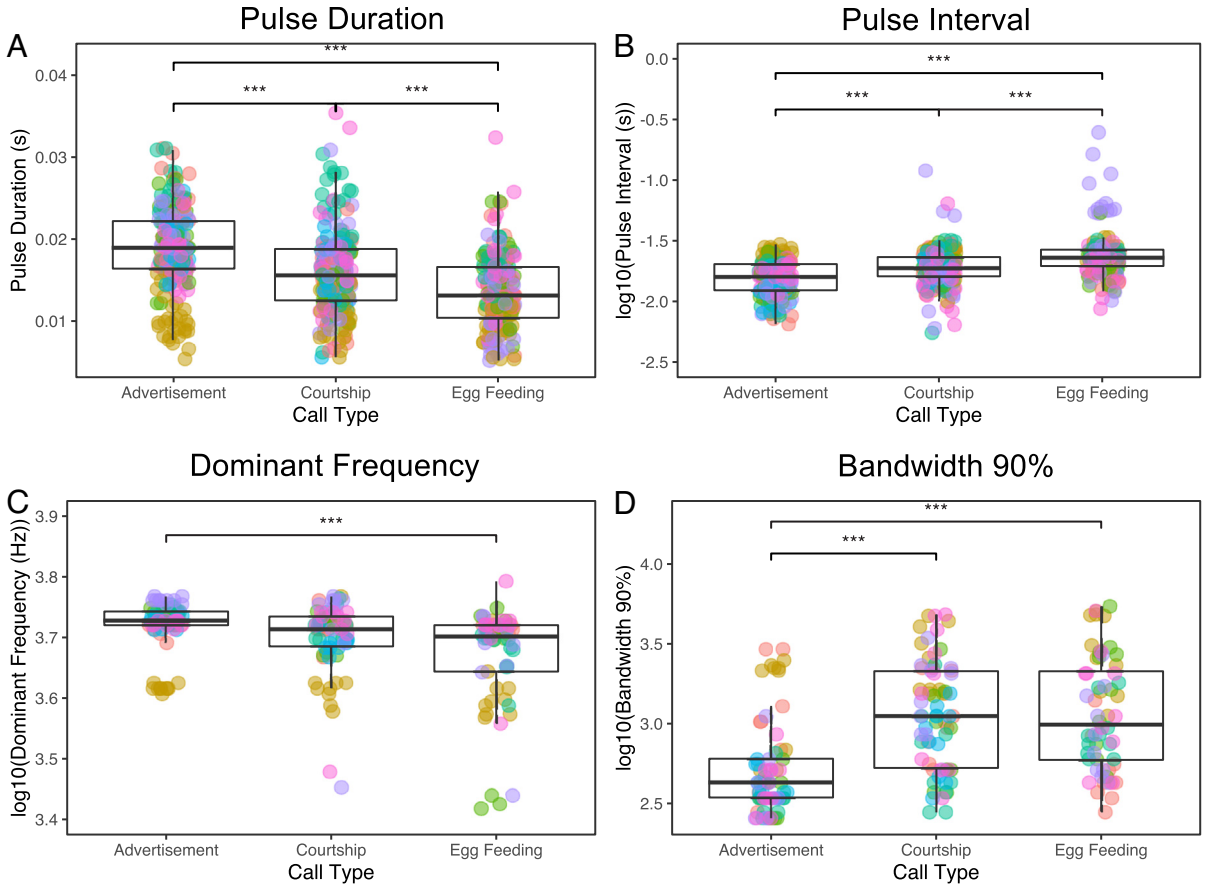

Fig. 3. Acoustic properties of *R. imitator* vocalizations vary between advertisement, courtship, and egg feeding calls. Plots depict distributions of four acoustic properties by call type: (*A*) Pulse duration; (*B*) log10 (Pulse interval); (*C*) log10 (Dominant frequency); and (*D*) log10 (Bandwidth 90%). Color of dots corresponds to seven unique males in the sample. Asterisks indicate significance levels for Tukey–HSD post hoc contrasts.

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. The highest accuracy of classification was for advertisement calls (82.9%) followed by courtship calls (73.5%) and 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 (*SI Appendix*, Fig. S4). Given that egg feeding calls were sampled at the same grain (10 calls from a single bout of egg feeding) for each

Table 1. Individual distinctiveness of nine acoustic properties of 70 advertisement calls, 68 courtship calls, and 56 egg feeding calls across seven males

	Advertisement			Courtship			Egg feeding		
Acoustic property	CV_a	Mean CV_w	PIC	CV_a	Mean CV_w	PIC	CV_a	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 (pulses/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

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 indicate that a call property varies more among individuals than within individuals (shown in bold).

male, variation in parental quality or specific parenting conditions may explain variation in call distinctiveness.

Discussion

Increased complexity of communication systems is predicted to co-evolve with the emergence of new forms of social interaction (1–3, 7), but this relationship has yet to be explored in the context of signals specialized for parental care. In this study, we characterized and compared calls of the biparental poison frog, *R. imitator*, across social contexts in which calls either serve an ancestral communication function—advertisement or courtship—or a derived social function—parental care via coordinated, trophic egg feeding. We found that vocalizations produced in distinct social contexts differ in both acoustic properties and the amount of identity information contained in those properties. 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, suggesting that egg feeding calls have undergone evolutionary modification in form to set them apart from ancestral signals. Despite this, nearly half of all egg feeding calls were not sufficiently distinct to be distinguished from ancestral call types based on acoustic properties alone, implicating a role for multimodal communication (e.g., visual, olfactory, tactile, and/or vibrational) in the coordination of parental behavior.

Studies of anuran acoustic communication recognize multiple common types of calls that function in distinct social contexts (16, 17). Advertisement calls are by far the most studied call type because they serve multiple social functions (i.e., mate attraction, territory defense, and species delimitation) and are therefore taxonomically widespread (17). Virtually no attention has been paid to characterizing vocalizations deployed during parental care, in which communication between the sexes holds a special significance beyond simple mate attraction. We quantified nine acoustic properties of vocalizations elicited during egg feeding in *R. imitator* and compared these to the same properties quantified for advertisement and courtship calls. Most properties of egg feeding calls were statistically indistinguishable from advertisement calls (i.e., call duration, number of pulses) or courtship calls (i.e., call bandwidth), consistent with the expectation that novel signals borrow heavily from ancestral elements that already function to elicit female responses. However, we also identified properties of egg feeding calls that were distinct from either ancestral call type (i.e., lower dominant frequencies and especially short pulse durations coupled with long pulse intervals) (Fig. 2*C*). Some of these differences in acoustic properties could be physiologically related to egg feeding calls being produced at lower amplitudes (i.e., "quieter") because pairs are typically in close proximity (<0.5 m) when males are leading females to tadpoles and stimulating them to feed (Movie S1). Systematic differences in amplitude between call types can introduce error into measures of spectral properties (36); however, such artifacts should result in narrower, rather than the broader bandwidths we observed for egg feeding calls, indicating our spectral measurements are robust. Moreover, courtship calls are also "quiet" close-range signals, so the unique elements of egg feeding calls cannot be entirely explained as physiological by-products of low amplitude calls. The modification of temporal patterning is a common theme in the evolution of context-dependent signals (e.g., refs. (37) and (38)) and we suggest the subtle differences observed in pulse durations and intervals reflect novel elements that are selectively maintained for the coordination of egg feeding.

A second major finding of our study was that the identity information conveyed in *R. imitator* vocalizations varies according to the social context in which they are deployed. By measuring calls repeatedly and across multiple social behaviors in the same males, we showed that advertisement calls contain the most individual identity information overall, with dominant frequency being the most reliable signature of male caller. Dominant frequency is relatively "static" within males of many species (e.g., refs. 39 and 40) presumably because it is constrained by the morphology of the sound-producing structures (41) and thus correlated with body size. Advertisement calls may be a signal or cue of individual identity used by rival males to recognize territory neighbors. In the case of mimic poison frogs, which are monogamous and pair bonding, individually distinctive advertisement calls could also be used by females to recognize their mate. If males benefit from being recognized by either territory neighbors or mates, selection could favor greater identity information in these calls to facilitate recognition. A complementary explanation is that courtship and egg-feeding calls are typically produced over shorter distances than advertisement calls, such that other signal modalities (e.g., visual, olfactory, tactile and/or vibrational cues) may interact with auditory cues in mediating individual recognition. Indeed, the majority of acoustic properties examined were more dynamic (i.e., showed more within-individual variation) in courtship and egg-feeding calls than in advertisement calls, which could reflect stronger selection for extreme values to enhance the motivational effects of these signals (39).

Finally, we evaluated whether subtle variation in the spectral range, temporal patterning, and individual distinctiveness was sufficient to quantitatively distinguish egg-feeding calls from ancestral call types. In a DFA, all three call types were classified with significantly greater than chance accuracy (i.e., >33%) from their acoustic properties alone; however, overlap between call types was considerable (Fig. 4) and calls elicited during egg feeding were misclassified in nearly half of all instances. Thus, despite their unique properties, vocalizations elicited during egg feeding may themselves be insufficient for conveying parental context. An intriguing observation can be made, however, in the patterns of misclassification. Egg-feeding calls in our sample were equally likely to be misclassified as advertisement or courtship calls, suggesting that egg-feeding calls borrow and recombine elements from multiple call types. Perhaps this complexity arises from the unique function of egg-feeding calls, which may align selection on the response with both advertisement (i.e., must attract females to a specific location) and courtship calls (i.e., must motivate females to lay eggs). Additionally, females deciding to lay trophic eggs (which are typically laid in the water) versus eggs that will be fertilized (which are typically attached to a leaf above the water line or in a location away from a pool) are likely responding to their own physiological state and other contextual cues as well as male signals. For one, tadpoles of this species beg (i.e., vibrate vigorously against parental frogs) to signal their hunger, which appears to be an honest signal of need important in eliciting trophic eggs (24). Use of multimodal cues would also be consistent with acoustic studies of other frogs, which have shown that behavioral responsiveness to male calls is enhanced when females are also presented with visual (42) or contextual cues (43). Future studies should focus on female perception of male call variants in relation to female state and other contextual cues to better understand how selection on signalers and receivers could synergistically drive the evolution of acoustic signals for trophic egg feeding.

In conclusion, our study examined acoustic signals produced in a parental coordination context in mimic poison frogs and contrasted these against ancestral signal types adapted to function in other facets of male-female communication. We found support for the prediction that greater complexity of the communication

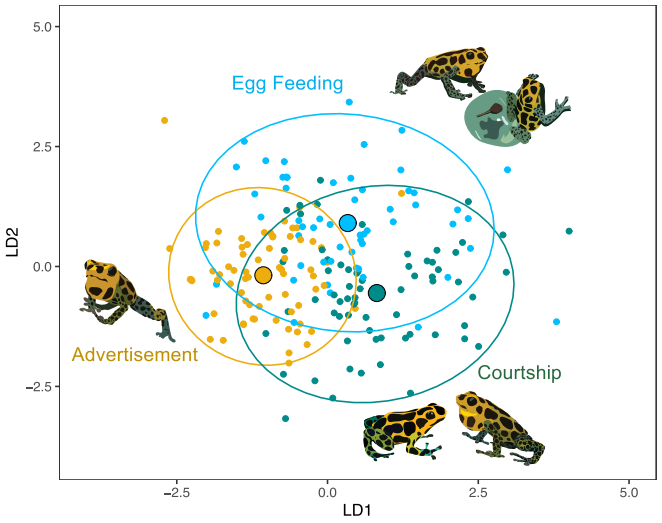

Fig. 4. Classification success of vocalizations based on acoustic properties exceeds chance levels, despite considerable overlap. Classification success was significantly above chance (33%) for all call types: 82.9% for advertisement calls, 73.5% for courtship calls (73.5%), and 57.1% for egg feeding calls. 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%). Plot of *R. 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% CIs (ellipses) depicted for each type.

system with the transition to biparental care with egg feeding involved the evolution of novel signal elements, likely via the recombination and modification of elements from ancestral signals. Our results highlight the complexity of anuran communication systems and the need to characterize vocal repertoires across a greater diversity of social contexts. More generally, this study contributes to understanding how communication systems are evolutionarily fine-tuned to convey context-dependent information in increasingly complex social systems. We hope that our approach may serve as a model for future investigations involving diverse taxa to explore the generalizability of these patterns.

Materials and Methods

Animal Husbandry. A captive colony of *R. imitator* descended from five localities in Peru (Veradero, Tarapoto, Huallaga, Sauce, Southern, and hybrids between these; *SI Appendix*, Table S1) was maintained in temperature- (71.86 ± 2.79 °C) and light- (12:12 h cycle) controlled rooms at the University of Illinois Urbana-Champaign (UIUC). Breeding pairs were housed in 12 × 12 × 18 inch glass terraria (©Exo Terra, Mansfield, MA, USA) containing live plants, driftwood, sphagnum moss, leaf litter, and water-filled film canisters for egg laying and tadpole deposition. Tank humidity (84.95 ± 3.08%) was maintained using a misting system (©Mist King, Ontario, Canada). Frogs were fed wingless *Drosophila* fruit flies dusted with vitamin supplements three times weekly. Only sexually mature individuals were used as focal subjects. All procedures were approved by the UIUC Animal Care and Use committee (Protocol #20147).

Acoustic Recordings. Between July 2021 and May 2022, seven male *R. imitator* were recorded in their home terraria across the breeding cycle. A shotgun microphone (K6/ME66, ©Sennheiser, Wennebostel, Germany) was positioned above the screen covering the focal tank and connected to a handheld recorder (H4n Pro, Zoom, Tokyo, Japan); 44.1kHZ sampling rate, 16-bit resolution. For each recording, we documented the time of day and reproductive stage of the focal male as: 1) nonbreeding (pair did not currently have eggs or tadpoles); 2) brooding (frog had at least one egg but hatching had not yet occurred); or 3) tadpole (at least one tadpole was present in a deposition pool). As *R. imitator* breed continuously and clutches are often overlapping, territory advertisement and courtship may occur at any reproductive stage; however, egg feeding occurs only in the presence of transported tadpoles. Thus, across all three stages, we distinguished advertisement calls from shorter courtship calls based on a published characterization of advertisement calls for this species (30). While advertisement calls produce a characteristic "trill" sound and function in long-range mate attraction and territory defense, courtship calls are typically shorter and softer and function in close-range interactions with females (29, 30). To isolate vocalizations elicited in the unique social context of egg feeding and test whether these can be quantitatively distinguished from advertisement and courtship calls, we took video recordings (RLC-510A, ©Reolink, New Castle, DE, USA) concurrently with audio recordings during the tadpole stage. Male *R. imitator* stimulate female partners to lay trophic eggs by leading them to tadpole deposition pools and maintaining close and/or tactile contact while calling continually (25). Thus, egg feeding calls were identified as calls coinciding with males moving toward tadpole deposition pools, leading females to pools, sitting on or near the pools, and culminating with the arrival of females at pools (e.g., Movie S1). Calls recorded during the tadpole stage while males were away from pools and/or oriented toward egg laying canisters were identified as either advertisement or courtship calls, based on the same criteria used during nonbreeding and brooding stages.

Call Filtering and Analysis. To examine variation within and among the three call types, ten calls of each type for each individual male were selected from available recordings (Dataset S2). Very low amplitude or low-quality calls, or calls that could not be clearly classified between the three types on the basis of social context or previous published accounts were excluded. Raw recordings of calls were trimmed in Audacity v. 3.0.2 (44). Analysis of calls was performed in Raven Pro v. 1.6.1 (31). In total, we measured nine acoustic properties that could be reliably quantified across all calls. We measured six temporal properties using the entire call or part of the call (i.e., a single pulse or interpulse interval) as the unit of measurement in the waveform view in Raven. These included call duration (time in seconds from the first pulse to the last pulse; *SI Appendix*, Fig. S2*A*), inter-call interval (time in seconds to the next call within the same bout, with bout defined as calls occurring within 1 min of each other; *SI Appendix*, Fig. S2*A*), number of pulses, pulse duration (time in seconds from the beginning of a pulse to the end; *SI Appendix*, Fig. S2*B*), pulse interval (time in seconds from the end of one pulse to the start of the next pulse), and pulse rate (the reciprocal of pulse period, or the full time from the beginning of one pulse to the beginning of next consecutive pulse; *SI Appendix*, Fig. S2*B*). Pulse duration, pulse interval, and pulse rate were calculated by taking the average of three pulses within the call. Because number of pulses was highly variable between calls, it was not possible to standardize pulse selection. Rather, we measured one representative pulse from the beginning, middle, and end of each call, non-sequentially. Because many calls showed abrupt (e.g., Fig. 2*B*) or gradual (e.g., *SI Appendix*, Fig. S1*A*) increases in amplitude over the first 1–6 pulses and we wanted to compare pulses of equivalent relative amplitude, introductory pulses at lower relative amplitude were excluded from pulse measurements. Large pulse intervals occasionally occurred at the beginning (e.g., Fig. 2*C*) or in the middle of a call. In these cases, pulses preceding large intervals were measured as one of the three pulses. Three spectral properties were also quantified using the entire call as the unit of measurement, including the dominant frequency of the call (in Hz; *SI Appendix*, Fig. S2*C*) and its bandwidth (90%; *SI Appendix*, Fig. S3*C*). In most cases, the dominant frequency corresponded to the second harmonic (5 to 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 to 4 kHz was observed and the harmonic structure was unclear (Fig. 2 and *SI Appendix*, Fig. S2). Thus, the frequency of the second harmonic was also quantified for each call.

Statistical Analyses. All statistical analyses were performed in R Studio v. 1.1.318 (45). 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" (46) and "emmeans" (47), 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 (intercall 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. We 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 PIC (47). 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; (34)), 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 PCA and all principal components were retained to calculate H_s (34). PCAs were implemented with the "prcomp" function in R 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 took a two-step multivariate approach. First, we performed PCA as described above. Input variables were prepared by converting acoustic properties into z-scores scaled to the mean of the individual, which controlled for variation among males. Second, principal components with eigenvalues > 1 were extracted as inputs into a DFA. We implemented this test using the linear discriminant analysis ("lda" function) in the package "MASS" (48). Chance-corrected classification success (Cohen's kappa) was calculated following the approach of (49) in the R package "vcd" (50). Differences in classification success among males were statistically evaluated using a Chi-squared test of independence.

Data, Materials, and Software Availability. We have uploaded our script and excised call files (.wav) to data dryad (https://datadryad.org/stash/share/Ks8yuD9AfUIxyFxQtqth6jzTBfDq3oIAbYszSrO24t8) (51). All other study data are included in the article and/or *SI Appendix*.

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