BRILL

Behaviour 151 (2014) 1313–1334

Behaviour
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Evolution of male and female release calls in African clawed frogs

Martha L. Tobias [a,*], **Jeremy Korsh** [a,b] **and Darcy B. Kelley** [a]

[a] Department of Biological Sciences, Columbia University, New York, NY 10027, USA
[b] Current address: Department of Orthopedics, UMDNJ,
Robert Wood Johnson School of Medicine, Newark, NJ 07107, USA
[*]Corresponding author's e-mail address: mt18@columbia.edu

Accepted 6 February 2014; published online 7 March 2014

Abstract
In anurans, male clasps can elicit release calls from either sex. Male release calls have been observed in many anuran genera and this vocal response is thus highly conserved. Female release calls, however, are not as prevalent, suggesting that evolutionary trajectories for anuran release calls differ by sex. We analyzed male and female release calls in all available species of *Xenopus*, a fully aquatic African genus. Phylogenetic relationships in this genus include three species groups, two of which are clades and one of which is characterized by a reticulated phylogeny due in part to hybridizations between species with different ploidy levels (Evans et al., 2004; Evans, 2008). In all species, males produce release calls when clasped by another male. Females in the reticulated group do not produce release calls, but females in the rest of the genus do. Release calls consist of click trains of variable durations and inter-call intervals. In both sexes, inter-click interval divides the genus into groups with different click rates and these groups are phylogenetically related. In general, inter-click interval is shorter in male than in female release calls. Across species and sexes, release calls are characterized by a single, low (∼1000 Hz) dominant frequency. In *X. laevis* Congo and *X. borealis*, clasp duration is longer for male–female than for male–male pairs and clasp duration is correlated with the number, but not the duration, of release calls in male–male pairs. We discuss evolutionary scenarios for release call traits as well as the sex difference in occurrence.

Keywords
vocal communication, *Xenopus*, dominant frequency, click rate, syntopy.

1. Introduction

Advertisement and release calls are the most common types of anuran vocalizations. Male advertisement calls attract conspecific females and can repel or silence male rivals (Blair, 1958; Brown & Littlejohn, 1972; Ryan

DOI:10.1163/1568539X-00003186

& Rand, 1995; Gerhardt & Huber, 2002; Tobias et al., 2004). Inappropriate sex partners, males and unreceptive females, produce a specific call type when clasped and this call can facilitate release from amplexus (Aronson & Noble, 1944; Marco & Lizana, 2002; Bowcock et al., 2008). Male release calls are widely conserved across species and genera, even in species that do not advertise (Di Tada et al., 2001; Marco & Lizana, 2002). Female release calls are expressed in both sexes of some genera (Kelley, 1982; McClelland & Wilczynski, 1989) but are notably absent in others (Di Tada et al., 2001; Marco & Lizana, 2002; Liao & Lu, 2009). The difference in conservation of release calls suggests that evolutionary trajectories for this vocal behavior may differ by sex, an hypothesis explored in this study.

That advertisement calls are essential for male reproductive success makes them a target for sexual selection, and the subject of extensive scientific investigation (reviewed in Gerhardt & Huber, 2002; see also Cocroft & Ryan, 1995). In contrast, release calls, which are not used for courtship directly, have received relatively little attention and much of that has focused on acoustic differences with advertisement calls (Sullivan & Wagner, 1988; Marquez & Eekhout, 2006; Garda et al., 2010). However, reducing the duration of clasping by an inappropriate partner is advantageous because prolonged amplexus incurs survival costs, increasing the risk of drowning and predation and decreasing locomotion and feeding opportunities (Verrell & McCabe, 1986; Sztatecsny et al., 2006; Bowcock et al., 2008, 2009). Release calls also contribute to reproductive success by liberating the clasping male to resume finding a mate and thus serve an important function during courtship. Cross-species similarity (convergence) of male release calls can facilitate call recognition between sympatric species. In European green toads (*B. viridis* complex), evolution of release calls in sympatric species is consistent with genetic drift rather than selection (Castellano et al., 2002). In Andean *Bufo*, sympatric species display one release call that is species-specific and another that is similar to heterospecific calls (Di Tada et al., 2001). A particular example of selection for similarity is convergent character displacement, in which sympatric species are more similar than their parapatric cohorts. Convergent character displacement for release call traits has been shown in some (Leary, 2001a) but not all (Leary, 2001b) sympatric *Bufo* species. Convergence has also not occurred in release calls from the hybrid offspring of *B. microscaphus* and *B. woodhousii* that are distinct from either parent species despite sympatry (Sullivan & Lamb, 1988). Here

we examine the extent of similarity between male release calls in *Xenopus*, comparing species that represent current instances of sympatry (Blackler et al., 1965; Picker, 1985; Fischer et al., 2000; Measey & Channing, 2003) to species whose geographic distributions are likely to preclude sympatry (from Tinsley et al., 1996). The genus *Xenopus* is characterized by species whose ploidy levels range from tetraploid to dodecaploid. These ploidy levels represent multiple instances of allopolyploidization due to inter-species hybridization (Evans, 2008) and thus in addition reveal ancient sympatry in *Xenopus*.

Prior studies of release call traits have been limited to comparisons between a few species or between the sexes in a single species. Here, we broaden our understanding of the evolution of this important behavior by surveying the occurrence and acoustic features of release calls in both sexes across all available species within the genus *Xenopus*. Because release calls can shorten amplexus, we also explore the correlation between release call and clasp durations in males and females in two species to evaluate call effectiveness in terminating a clasp. The genus *Xenopus* provides significant advantages for a study of this scope. A large number of captive species and populations of *Xenopus* are available for recording and behavioral experiments. *Xenopus* are entirely aquatic with murky ponds as a preferred habitat. Thus, while some terrestrial frogs combine visual and acoustic signals in mate attraction (e.g., Narins et al., 2003), the auditory modality is paramount during *Xenopus* courtship (Picker, 1980; Tobias et al., 1998, 2004). The molecular phylogeny of the genus provides an evolutionary framework (Evans et al., 2004; Evans, 2008) for the comparison of release calls across species and sexes.

2. Materials and methods

2.1. Experimental approach, animals and recording

In *X. laevis* South Africa, release calls cannot always be evoked artificially by grasping (as is usual for other anurans) but do reliably accompany clasping by an actual male, the behavioral paradigm used here. Release calls are typically elicited during every clasp. For example, in the two most extensively examined species in this paper, *X. l.* Congo and *X. borealis*, release calls were elicited in 100% of clasps. Before accepting the null hypothesis,

that a species did not release call, release calling had to be absent from at least 3 individuals/species clasped at least 3 times each.

For experiments to characterize acoustic properties, release calls made by the clasped male or female were recorded with a small hydrophone (output sensitivity −52 dB, 0.1–6 kHz; Knowles, Itasca, IL, USA) fixed to a Lucite rod, placed next to the clasped animal's head. A second hydrophone (High Tech, Gulfport, MI, USA; output sensitivity −164.5 dB at 1 V/μPa, frequency sensitivity 0.015–10 kHz) was placed in the tank and recorded both clasping and clasped frogs' calls. Comparison between the two recordings is a reliable means of identifying which frog produced a given call (see Tobias et al., 2004 for details). All calls were recorded on CD or flash card (CDR300, Marantz, Mahwah, NJ, USA; 44.1 kHz sampling rate) or on a computer (MacIntosh) via a Lexicon A/D converter. Release calls were then selected from the entire recording and band-pass filtered (between 200–3000 Hz) using Goldwave (St. John's, NF, Canada) or Amadeus software (Hairersoft). *Xenopus* release calls are composed of clicks, made when the laryngeal arytenoid discs open, and are analogous to a pulse in terrestrial frog calls. Call characters, inter-click interval and spectral frequency, were subsequently analyzed using Signal software (Engineering Design, Berkeley, CA, USA) running on a Dell (Microsoft Windows XP) or Amadeus on a MacIntosh (Apple OSX). Inter-click interval is the time between the onset of one click and the onset of the succeeding click. ICIs were calculated manually from waveforms. Spectral frequency was measured from power spectra of the waveform of an entire release call (transform length = 32 768 points, resolution = 743 ms (1.3 Hz) using a Hanning window). Waveform amplitude is given in volts and spectrograms (amplitude of different frequency components in a call) in dB.

We recorded release calls from 16 species and 5 populations of *Xenopus*, all resident in our laboratory. The origins of species and populations have been described (Tobias et al., 2011). These species and populations are representative of all captive populations outside of Africa. Species that were not available to record from have been omitted from Figure 1. Whenever possible, release calls from three individuals per species were recorded. The number of animals recorded per species and the number of calls per animal are indicated in Table A1 (Appendix). Recording sessions lasted as long as males attempted to clasp the other frog or until the unreceptive frog had been clasped 4 times.

Animals were group housed according to size (3–5 per tank for larger species, 6–10 per tank for smaller species) in polycarbonate tanks (43 × 29.5 × 19.5 cm, l × w × h), filled 2/3 with dechlorinated, filtered water. Frogs were maintained on a 12/12 light dark cycle at 21°C and fed three times/week. Individual, uninjected test males or females were paired with a male injected with gonadotropin (hCG; Sigma; 50–200 IU depending on body size) the day before and day of testing to promote clasping; experimental pairs were recorded in a 10 gallon glass aquarium, separated from all other animals.

To measure the correlation between release call and clasp duration and to compare the efficacy of release calling across species and sex, we analyzed release calls produced during all clasps in two species. Pairs included a receptive male (hCG injected as described above) and either an unreceptive male or female. The experimenter's notation of clasp onset and termination was recorded on one channel of the computer and the animal's release calls on the other channel. Clasp duration was subsequently calculated from the recording and compared to the duration of release calling. Since release calls had been previously analyzed (see above) and were thus recognizable, only one hydrophone was required for this experiment. In these experiments, recording sessions lasted until the receptive male stopped clasping.

2.2. Statistics

A *t*-test was used to compare release and clasp durations between species. A two-tailed *t*-test with a Welch correction for unequal variances was used to compare male vs. female dominant frequency and inter-click interval (ICI) within a species (using a mean for each animal). To determine whether click rate fell into distinct categories, we first graphed the means for each species and observed that these fell into fast and slow groups. We then confirmed that group means were significantly different using a two-tailed *t*-test. A Mann–Whitney test was used when there were unequal variances in the two groups being compared or when the measure was non-parametric, i.e., clasp duration in males and females and % time spent calling during a clasp in *X. borealis* and *X. l.* Congo males. A Spearman correlation was used to compare clasp duration with number of release calls.

We identified species in which syntopy (residence in the same pond) has been reported (Blackler et al., 1965; Picker, 1985; Fischer et al., 2000; Measey & Channing, 2003) and species whose geographic distributions are

likely to preclude syntopy (from Tinsley et al., 1996) and compared release call characteristics for syntopic pairs. To assess ICI differences between each syntopic and parapatric pair, each release call (rather than means) from each species was used because the number of available individuals was sometimes less than three (Mann–Whitney test).

3. Results

3.1. Phylogeny of male and female release calls

The presence of release calls in response to clasps was examined in both sexes in all available species of *Xenopus*. The *Xenopus* genus is divided into three major species groups and two extra-cladal species (*X. largeni* and *X. clivii*, Figure 1). The species groups are: the *borealis* and *laevis* clades, in which extant species share a common ancestor (all tetraploid), and the reticulated group, in which extant species share more than one ancestor. The reticulated group includes all the higher ploidy (octo- and dodecoploid) species, many of which result from inter-species hybridization events (Evans, 2008).

Male release calls were recorded in every species examined while female release calls were recorded from only some species and these are phylogenetically related (Figure 1). Female release calls were recorded from all species in the *borealis* and *laevis* clades, as well as in *X. clivii*. All species that did not exhibit female release calls were found in the reticulated portion of the phylogeny.

3.2. Male and female release call traits

Each click in a male release call contained a broad band of frequencies with a dominant peak (Figure 2A, B). Male release calls from *X. l.* South Africa and *X. borealis* are illustrated in Figure 2C–F. The *X. borealis* spectrogram (Figure 2D) indicates that the dominant frequency corresponded to the sustained portion of the click (Figure 2B). Each release call contained multiple clicks with a consistent inter-click interval, or click rate; *X. l.* South Africa had a faster click rate than *X. borealis* (Figure 2E, F).

Figure 3 illustrates female release calls from two species that differed in click rate. As for males, female release call clicks were broadband, with the dominant frequency corresponding to the sustained portion of the click (Figure 3A–D) and inter-click interval was consistent throughout the release call (Figure 3E, F).

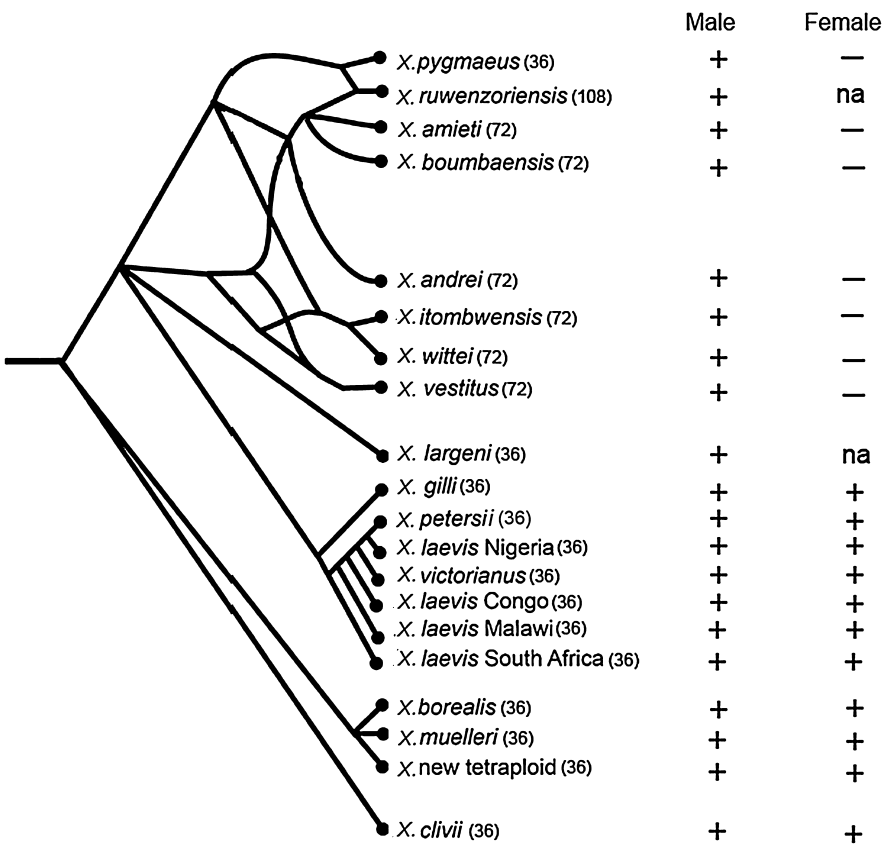

Figure 1. Occurrence of release calls across an estimated molecular phylogeny for *Xenopus* for all available species (na indicates species in which females were not available). Species' ploidy level is shown in parentheses. Species and population nomenclature according to Tobias et al. (2011).

The dominant frequency of male release calls was typically low; the mean ± SD for all species was 1009 ± 268 Hz (Figure 4A). The one exception was *X. boumbaensis* whose release calls contain a higher and more variable dominant frequency (1908 ± 501 Hz); the mean ± SD for all species excluding *X. boumbaensis* is 961 ± 169 Hz. The generally low pitch of male release calls gave rise to their original moniker, "growling", for *X. l.* South Africa (Picker, 1980). Although there were significant species differences in frequency, for example between *X. amieti* and *X. andrei*, dominant frequency overlapped extensively across species. The dominant frequency of female re-

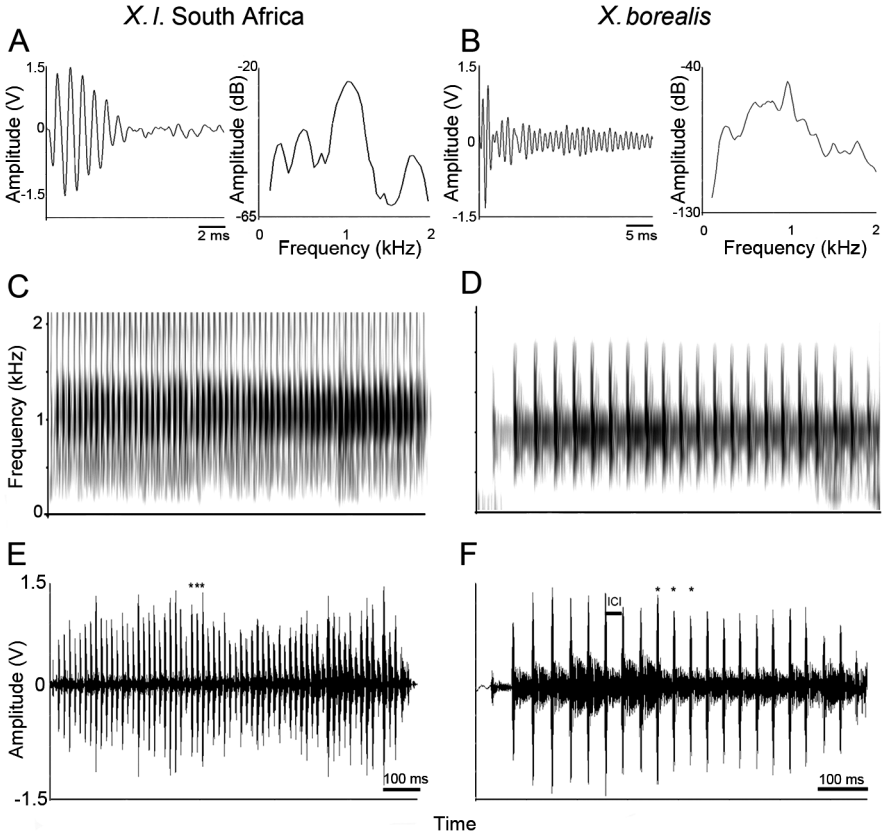

Figure 2. Male release calls from two *Xenopus* species with fast (*X. l.* South Africa) and slow (*X. borealis*) click rates. (A) At left, waveform of a single click for *X. l.* South Africa; at right, power spectrum for that click. (B) Click waveform (left) and power spectrum (right) for *X. borealis*. Spectrogram (C) and waveform (E) of a *X. l.* South Africa release call. Spectrogram (D) and waveform (F) of a *X. borealis* release call. Asterisks over 3 consecutive clicks.

lease calls was also low (871 ± 79 Hz) and broadly overlapped across species (Figure 4B).

Inter-click interval, in contrast, divided the phylogeny into groups (Figure 4C, D). For males (Figure 4C), click rate can be fast (ICI 15.2 ± 3.1 ms, including *X. pygmaeus*) or slow (ICI 41.6 ± 4.2 ms), and these rate groups differed significantly ($t = 14.4$, df $= 18$, $p < 0.0001$). Species with similar rates were phylogenetically related (e.g., faster in the *laevis* and reticulated species groups and slower in the *borealis* clade) with the exception of *X. boumbaensis*, which had a slow click rate but is genetically more closely

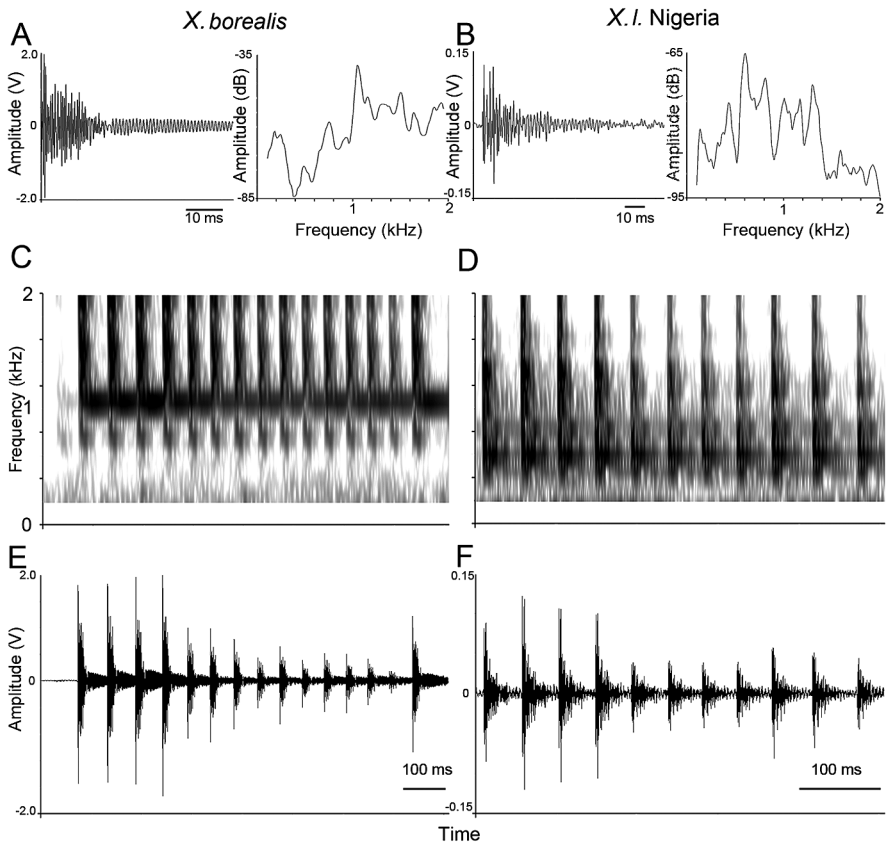

Figure 3. Female release calls from two species with fast (*X. borealis*) and intermediate (*X. l.* Nigeria) click rates. (A) Waveform (left) and power spectrum (right) of a single click from *X. borealis*. (B) Waveform (left) and power spectrum (right) for a *X. l.* Nigeria click. Spectrogram (C) and waveform (E) for one *X. borealis* release call. Spectrogram (D) and waveform (F) for one *X. l.* Nigeria release call. Note that the dominant frequencies in the power spectra (A, B) correspond to the sustained portion of the click (C, D).

related to species with faster click rates. For females, there were three discernible click rate groups and these were also phylogenetically related (Figure 4D). In the *borealis* clade and *X. clivii*, ICIs were shortest (50.6 ± 5.1 ms). In the *laevis* clade, ICIs were either intermediate in length (103.3 ± 11.6 ms) or long (219.7 ± 1.2 ms). Values for the short and intermediate rate groups were significantly different ($t = 8.4$, df $= 7$, $p < 0.0001$). The ICIs for the slow group were not compared statistically as there were only two species, but the mean ± SD for this group did not overlap the fast or in-

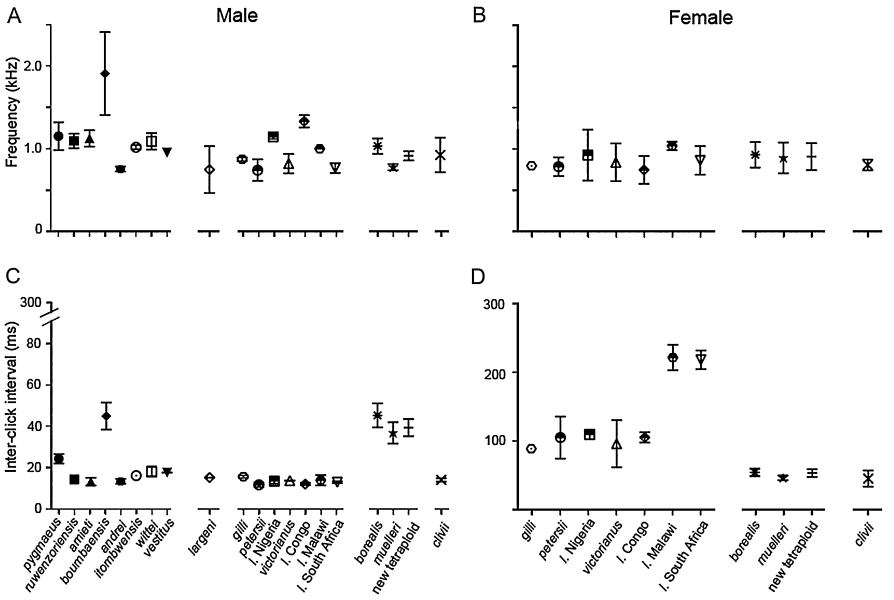

Figure 4. Dominant frequency and click rate (inter-click interval) for all *Xenopus* male and female release calls. Symbols are unique to each species (x-axis) and error bars denote mean ± SD. Genus name has been removed for clarity. (A, B) Dominant frequency is similar across species and sexes with the exception of *X. boumbaensis* in males. (C) Inter-click interval in male release calls divides the genus into a fast click rate group, that includes most species, and a slower click rate group, that includes 4 species. (D) Inter-click interval divides female release calls into slow, intermediate and fast click rate groups. For both sexes, rate groups are phylogenetically related; x-axis is ordered from left to right as in the phylogeny in Figure 1. Clades/species group are/is depicted by continuous lines on the x-axis.

termediate groups. There was extensive overlap within rate groups for both sexes.

There was no sex difference in dominant frequency for any species (Figure 5A). In contrast, inter-click interval was longer on average in females for all species although the difference was least pronounced in the *borealis* clade (Figure 5B). The sex difference in mean ICI was significant in all but three species, and two of these are in the *borealis* clade (Figure 5B).

3.3. Syntopy and male release call traits

If heterospecific communication favors similarity in release call traits, we might expect more similar values in syntopic (sharing a body of water) than in parapatric (geographically non-overlapping) species. Although the benefit of cross species communication could apply to either sex, we could only

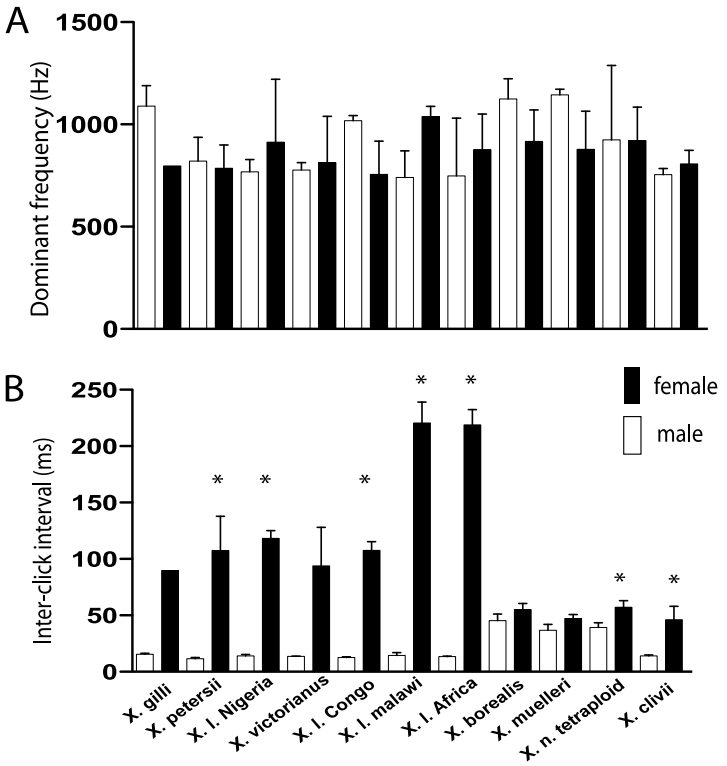

Figure 5. Comparison of release call acoustic traits between the sexes. (A) Dominant frequency (mean ± SD) for each species. There is no significant sex difference in dominant frequency in any species. (B) Inter-click interval (mean ± SD) for each species. Inter-click interval is significantly greater in females for all species except for two members of the borealis clade, *X. borealis* and *X. muelleri* and in *X. victorianus*. Data from *X. gilli* were not analyzed statistically as only one female was available. Asterisks represent $p < 0.05$.

compare male release calls because most of the syntopic species do not exhibit female release calls. We compared ICI and DF in 7 reported syntopic species pairs (Table 1; see Methods for references). Of the pairs in which both species have short ICIs (first 5 pairs in Table 1), mean ICI was significantly different in three. Mean ICIs in the two syntopic pairs that included species with both a fast and a slow ICI were also significantly different. DF was significantly different in 6/7 syntopic pairs. Similarity in ICI and DF was next compared in parapatric species in which reported geographic distances between the species are great enough to preclude sympatry (determined from geographic distribution maps in Tinsley et al., 1996). Differences

Table 1.
Release call differences in syntopic and parapatric species pairs.

	Mean ICI difference (ms) ($p <$)	Mean DF difference (Hz) ($p <$)	Genetic distance
Syntopic species			
l. South Africa/*gilli*	1.9 (0.0001)	303 (0.02)	0.027
l. South Africa/*vestitus*	4.3 (0.0001)	320 (0.01)	0.054
l. South Africa/*wittei*	5.9 (0.0003)	428 (0.04)	0.055
clivii/*largeni*	0.2 (NS)	128 (0.001)	0.084
vestitus/*wittei*	1.6 (NS)	108 (NS)	0.037
borealis/*victorianus*	27.1 (0.0001)	345 (0.0001)	0.084
l. South Africa/*muelleri*	25.3 (0.0001)	396 (0.003)	0.084
Parapatric species			
amieti/*andrei*	0.5 (NS)	70 (NS)	0.03
l. Nigeria/*andrei*	0.8 (NS)	352 (0.0001)	0.055
gilli/*amieti*	2.2 (0.014)	122 (0.05)	0.049
clivii/*gilli*	0.01 (NS)	303 (0.0001)	0.084
l. Nigeria/*victorianus*	0.3 (NS)	30 (0.05)	0.002
clivii/*l.* South Africa	2.0 (0.003)	0.5 (NS)	0.084

were measured in 6 parapatric species pairs all belonging to the fast ICI group. Mean ICI is significantly different in 2/6 pairs and mean DF is significantly different in 4/6 pairs. Thus, similarity was, if anything, more prevalent in parapatric than in syntopic pairs. The mean ICI difference was small but significantly greater in fast syntopic than in fast parapatric pairs (3.1 ± 0.6 vs. 1.1 ± 0.4; $t = 2.8$, df $= 9$, $p < 0.02$); mean DF did not differ between syntopic and parapatric pairs. The data thus did not support convergence of release call traits in syntopic species.

3.4. Release call and clasp durations

More than one release call was made during a single clasp (Figure 6). For both sexes, release calls within a clasp were variable in duration and intercall interval. Because release calls were only produced during a clasp, one possibility was that release call duration was determined by clasp duration. We compared male release call duration to clasp duration for two species, one from the slow click rate (*X. borealis*) and one from the fast click rate (*X. l.* Congo) groups (Figure 7). Release calls were shorter in *X. l.* Congo than in *X. borealis* males (0.7 ± 0.5 vs. 1.8 ± 1.4 s, $t = 8.7$, df $= 147$, $p < 0.0001$)

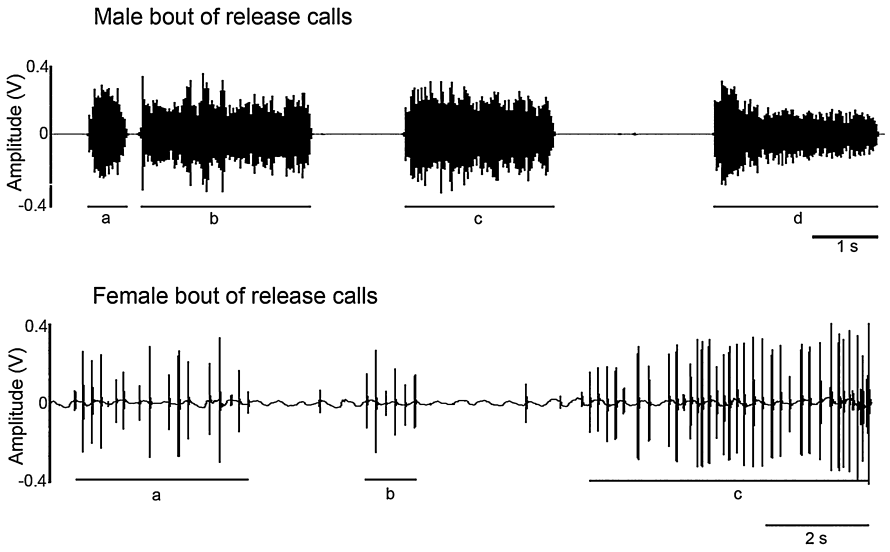

Figure 6. Bouts of release calling for a male and a female *X. l.* Congo during a single clasp. Successive release calls (indicated by letters) illustrate their variable duration and inter-call interval.

and clasps were longer (42.1 ± 26.1 vs. 15.8 ± 13.8 s, $t = 10.92$, df = 397, $p < 0.0001$; Figure 7A, B). However, clasp and release call durations were highly variable in both species. For example, clasp duration in *X. borealis* varied from 1 to 54.4 s and release call duration varied from 0.2 to 6.5 s in a 22 s clasp. Since short and long release calls occurred within the same clasp, clasp duration did not determine release call duration. Clasp duration was however significantly correlated with number of release calls for both species (*X. l.* Congo: $r = 0.84$, $p < 0.0001$, $N = 31$; *X. borealis*: $r = 0.73$, $p < 0.0001$, $N = 84$; Figure 6C, D). Thus, longer clasps resulted in more, but not longer, release calls.

We next considered whether release calls of one species were more effective in terminating a clasp than in another. We reasoned that if the species with shorter clasps, *X. borealis*, also spent less time release calling during a clasp, this result would indicate greater effectiveness. However, *X. borealis* males spent a significantly greater proportion of time release calling during a clasp than did *X. l.* Congo (57.9 ± 25 vs. 38.0 ± 24%; $U = 230$, $N = 26$ (*X. borealis*) and 32 (*X.* Congo), $p < 0.0037$). Thus, although the greater amount of time spent release calling may have reduced clasp duration in *X. borealis*, it does not indicate a more efficacious call.

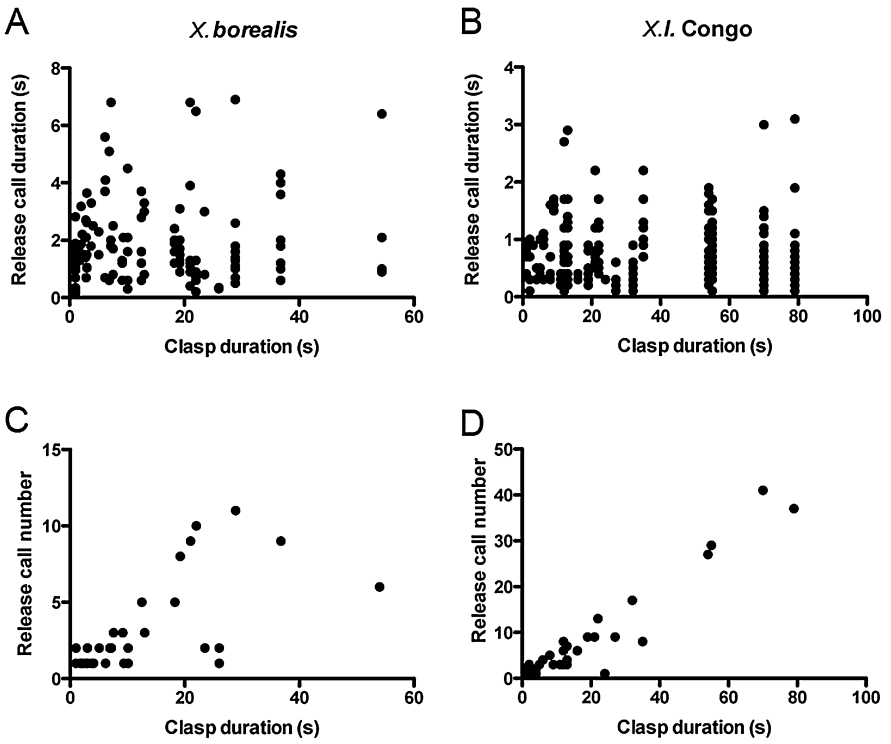

Figure 7. Release call durations and numbers for *X. borealis* and *X. l.* Congo male clasps. (A, B) Release call duration is highly variable and not correlated with clasp duration for either species. (C, D) Release call number is positively correlated with clasp duration for both species.

The average duration of clasps in male–female pairs was 88.4 ± 48.0 s in *X. borealis* and 311.3 ± 316.5 s in *X. l.* Congo. For both species, clasp durations were significantly longer for male–female than for male–male pairs (*X. borealis*: 15.8 ± 13.8, $U = 1920$, $N = 291$ (female) and 134 (male), $p < 0.0001$; *X. l.* Congo: 42.1 ± 26.1, $U = 6377$, $N = 104$ (female) and 267 (male) $p < 0.0001$). For both sexes, clasp durations were longer in *X. l.* Congo. Since clasps might be longer if females produced less release calling, we also examined the percentage of time spent release calling during a clasp. The proportion of time spent release calling was significantly greater in male–female than in male–male *X. l.* Congo pairs ($t = 3.6$, df = 58, $p < 0.0007$; 61.1 ± 26.3 vs. 38.0 ± 23.7%, respectively). That clasps were longer despite more release calling indicates that female release calls were less effective than male release calls at terminating clasps in *X. l.* Congo. In

X. borealis, although there was no difference in the proportion of time spent release calling during male–male and male–female clasps ($t = 1.7$, df $= 41$, $p = 0.1$; mean $\pm$ SD: males $= 57.9 \pm 25\%$, females $= 45.2 \pm 22.7\%$), clasp duration was longer in male–female pairs again suggesting that female release calls were less effective than male release calls.

4. Discussion

4.1. Evolution of male and female release calls and the role of female release calls

Release calls across available *Xenopus* species were surveyed to examine the evolution of this behavior for each sex. While male release calls were recorded in all species, female release calls were recorded only from the laevis and borealis clades as well as from *X. clivii* (11 species/populations), but not from the reticulated group (7 species). This observation suggests that the ancestral state included female release calling since it is present in two clades with shared ancestry. In this scenario, female calls were lost in the reticulated group after divergence (approx. 12 million years ago) from the most recent common ancestor shared with the *laevis* clade (Anderson & Evans, 2009).

While the coincidence between the loss of release calls and the high incidence of hybridization in the reticulated species group is striking, it is unlikely that the loss contributed to hybridization. The female release call signals lack of sexual readiness, i.e., no mature, ovulated eggs in the oviduct available for oviposition. Because sexually unreceptive females cannot provide eggs for males to fertilize, loss of the release call is not advantageous for hybridization.

That males in all species produce release calls when clasped suggests that this call type serves a vital function, probably related to the reproductive disadvantage of time and effort spent clasping a male rather than a female. Rapid release from amplexus in male–male pairs spares both partners time and energy for locating and mating with the other sex. Male release calls are strongly conserved in anurans generally, even in species in which males do not vocally advertise (Di Tada et al., 2001; Marco & Lizana, 2002), supporting their importance in social communication.

In contrast, female release calls are not found in all anuran genera, being notably absent in many species of *Bufo* (Di Tada et al., 2001; Marco &

Lizana, 2002; Liao & Lu, 2009) and, as reported here, in many *Xenopus*. As for male–male pairs, clasping a sexually unreceptive female is disadvantageous for each partner due to wasted effort on the part of the male and hindrance in moving, feeding (and even breathing) for both sexes (Bowcock et al., 2008, 2009). However, the costs of male–male versus male–female amplexus differ: effort expended on amplexus with a male will have an unfavorable reproductive outcome for both partners while amplexus with a female could be advantageous if she is on the cusp of receptivity (Measey & Tinsley, 1997).

In bufonids, females of many species are incapable of sound production (Schmidt, 1972; Marco & Lizana, 2002; Bowcock et al., 2008; Liao & Lu, 2009). Females in the reticulated species group of *Xenopus* that do not produce release calls are not necessarily mute. We have recorded a receptive call from one female member of the reticulated species group, *X. amieti* (U. Kwong-Brown, M. Tobias & D. Kelley, unpublished observations), suggesting that at least some females in this group can vocalize even though they do not produce a release call. However, sexually unreceptive females can effect release using non-vocal signals. One example is body vibration used by *Bufo* females to signal an unreceptive state (Blair, 1947; Bowcock et al., 2008). In *Xenopus*, *X. l.* South Africa and *X. wittei* females release call and extend their hindlegs, impeding the male's inguinal clasp (Weintraub et al., 1985; Measey & Tinsley, 1997). One hypothesis arising from this study is that females from the reticulated species group use an alternative method of conveying lack of receptivity. Females can also avoid the disadvantages of prolonged amplexus by dislodging the male by struggling or fighting. Whether this behavior is novel or an exaggerated form of a behavior (i.e., leg extension, struggling) shared with species that do release call remains to be examined. Preliminary data suggest the latter; observations of clasped females from one species in the reticulated species group (*X. amieti*) and one in the *laevis* clade (*X. l.* Congo) indicate that these species differed in the amount of time that females are quiescent during a clasp. While most females capable of release calling were idle for some time during a clasp, females that did not release call, either extended their legs or struggled, but were rarely idle.

Why then do any females release call? Females have poor choices when it comes to saying "no". In *Xenopus*, females can either release call, which verifies their sex, or remain silent, an ambivalent signal that also conveys receptivity (Weintraub et al., 1985; Wu et al., 2001) and either vocal behavior

may encourage a longer clasp. In addition, female release calls may attract nearby males resulting in multi-male clasps that incur increased survival costs. Although the duration of male-unreceptive female clasps are longer on average than male–male clasps, clasps are considerably shorter compared with male-receptive female clasps that can last hours in *Xenopus* (Kelley, 1982) or days in other anurans (Wells, 1977). Thus, release calls may simply be a more energy efficient means of effecting release.

4.2. Role of sex differences in acoustic features of the release call

Acoustic differences between male and female release calls suggest that they might differ functionally with respect to terminating amplexus. Reports comparing the duration of amplexus in species in which both sexes produce a release call are rare. In *Rana pipiens*, male–male amplexus is of much shorter duration than male–female amplexus despite the presence of release calls in both sexes; male release calls produce release since clasp duration in muted males is longer than in calling males (Aronson & Noble, 1944). For *Xenopus*, male–male clasps are also shorter than male–female clasps in two species in which both sexes produce release calls. In both species, clasp durations were greater in male–female pairs whether or not the proportion of time spent release calling was more than in male–male pairs, suggesting that female release calls are less effective than male calls in terminating amplexus.

Given that clasp durations differ depending on the sex, the temporal or spectral features of female release calls can be used to distinguish the sex of a partner. Sex differences in spectral properties are evident in fire bellied toads (Bombinatoridae; Gollman et al., 2009), *Rana temporaria* (Brzoska et al., 1971) and *Rana pipiens* (McClelland & Wilczynski, 1989). Sex differences in temporal features can accompany spectral differences, as in *R. temporaria* and *R. pipiens* (Brzoska et al., 1971) or be the only sex difference, as in *R. catesbiana* (Boyd, 1992). *Xenopus* release calls are monotonous trills of variable duration in both sexes. In most species in which both sexes release call, click rate is significantly slower in females. Dominant frequency does not differ by sex. Thus, the primary acoustic feature available for sex recognition from release calls is inter-click interval.

Species groups in *Xenopus* differ in the dissimilarity of release call rates between the sexes. In the borealis clade, female click rates are more similar to those of males than in the laevis clade. This difference may be due to physiological constraints imposed by muscle fiber properties in the vocal

organ. In *X. l.* South Africa, female laryngeal muscle is composed primarily of slow twitch fibers while male laryngeal muscle is composed entirely of fast twitch fibers (Sassoon et al., 1987); thus, females are incapable of rapid click rates (Tobias & Kelley, 1987). This is not the case in *X. borealis*, in which laryngeal muscle contains both fast and slow twitch types in both sexes (Leininger, Kitayama & Kelley, unpublished), a feature that imposes limits on sex differences in release call click rate.

4.3. Evolution of acoustic features in male release calls

The low variation in dominant frequency of male release calls between *Xenopus* species suggests that the evolution of this trait is consistent with genetic drift or that the rate of evolution is low. Dominant frequency of female release calls is not significantly different from males within a species and is overlapping across species. A low frequency release call is not unique to the underwater vocal life of *Xenopus*. Release call frequency in bufonids is similar to *Xenopus* and even lower in ranid males and females (Sullivan & Wagner, 1988; McClelland & Wilczynski, 1989; Boyd, 1992; Castellano et al., 2002; Bowcock et al., 2008), suggesting that low frequency release calls are conserved across anuran groups. Low frequencies in male release calls could be used to distinguish these from other call types (McClelland & Wilczynski, 1989; Gollman et al., 2009). In *Xenopus*, the dominant frequency is always lower in release than in advertisement calls across species (compare with Tobias et al., 2011). The widespread preservation of low frequency release calls across sexes and genera suggests that this acoustic feature may be subject to stabilizing selection that limits variation.

Across the genus *Xenopus*, the male vocal organ is capable of producing clicks with spectral features that vary according to call type: a broadband, lower frequency for release calls (this paper) and two dominant frequency peaks for advertisement calls (Tobias et al., 2011). The difference in spectral features of these two call types is thus an ancient and conserved feature of vocal communication in the genus. The clicks in release and advertisement calling result from contraction of laryngeal muscles driven by the activity patterns of vocal motor neurons in the hindbrain (e.g., Tobias & Kelley, 1987; Yamaguchi & Kelley, 2000). The spectral qualities of calls thus are not an inevitable by-product of laryngeal physiology but are controlled instead by the output of the central nervous system and represent targets for selection.

Click rate in *Xenopus* has had a more dynamic history of evolutionary change than dominant frequency. Click rate divides the phylogeny into two

groups with high within group similarities and large between group differences (range in the fast rate group is 11.7 to 18.1 ms with a mean difference of 6.4 ms; range in the slow rate group is 36.8 to 44.9 ms with a mean difference of 8.1 ms). The species exhibiting the slow rate include all members of the *X. borealis* clade and one distantly related species, *X. boumbaensis*. This pattern suggests that click rate diverged from fast to slow early in *Xenopus* evolution with the emergence of the borealis clade, and was maintained after it emerged. This was followed by a secondary, isolated divergence in *X. boumbaensis*. That slow click rate occurs in distantly related species in the genus is, like advertisement call type (Tobias et al., 2011), an example of homoplasy.

Acknowledgements

We wish to thank Ben Evans for helpful comments on the manuscript and for supplying genetic distances between species. Further thanks to Zach Bell and Fady Eid for assistance with experiments. Work in the authors' laboratory is supported by NIH grant NS23684.

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Appendix

Table A.1.
Numbers of animals/species and calls/animal.

Species	No. of calls	Species	No. of calls	
	Male		Male	Female
X. pygmaeus	12	*X. gilli*	3	1
	6		11	
	3	*X. petersii*	1	6
X. ruwenzoriensis	1		7	9
X. amieti	1		2	3
	5		7	
	2	*X. l.* Nigeria	7	7
	1		4	5
	4		3	3
X. boumbaensis	1		5	
	8		1	
	10	*X. victorianus*	11	8
	1		21	6
	3		5	12
X. andrei	4	*X. l.* Congo	3	2
	17		7	5
	6		4	6
	7	*X. l.* Malawi	6	1
	4		2	5
X. itombwensis	4		3	5
X. wittei	1			3
	2	*X. l.* South Africa	4	7
	1		4	3
X. vestitus	10		4	5
	3	*X. borealis*	6	2
X. largeni	2		2	12
	3		6	3
			2	
		X. muelleri	5	4
			7	2
			3	3
		X. new tetrapolid	4	1
			4	1
			4	2
				1
		X. clivii	9	10
			3	5
			3	3

Each row represents one frog.