Signal Synchrony and Alternation Among Neighbor Males in a Japanese Stream Breeding Treefrog, *Buergeria japonica*

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Abstract: Animals that aggregate in leks to attract mates often time the production of their mating signals against the signals of neighboring conspecifics. Such signal timing usually falls into general patterns within these aggregations, which can be categorized based on the amount of overlap between the signals. In many species, individuals produce signals in an alternating pattern, avoiding signal overlap to reduce interference and increase mate attraction. In contrast, individuals in some species produce signals in synchrony, maximizing overlap and interference. The prevalence and function of signal synchronization is still unknown in many species. Here we examine the call timing strategies of the Ryukyu Kajika frog (*Buergeria japonica*). Using acoustic playback experiments we characterize a divergence in timing patterns between the two call types in this species, one produced in alternation and one in synchrony. Specifically, male *B. japonica* responded to playbacks of the first call type (Type I calls) with delayed Type I calls, avoiding overlap with the playbacks. In contrast, males responded to playbacks of the second call type (Type II calls) with synchronized Type II calls, overlapping their calls with the playbacks. Such variation in temporal signaling strategies within a species provides insights into how social and environmental pressures shape signal timings.

Key words: Acoustic communication; Audio playback; *Buergeria japonica*; Call timing; Synchronized signals

**INTRODUCTION**

Many animals aggregate in large groups when producing mating signals. In such aggregations, signals produced at the same time can interfere with one another, reducing the ability of receivers to discriminate between individual signals (Gerhardt and Klump, 1988; Wollerman, 1999). This challenge of communicating in groups is most notable in the leks of many insect and anuran species, where female receivers select a preferred
signaling male from aggregations of sometimes hundreds of signalers (Gerhardt and Huber, 2002). Indeed, there are numerous behavioural adaptations for group communication (Brumm and Slabbekoorn, 2005; Bee and Micheyl, 2008). One strategy is for signalers to avoid overlapping the timing of signal production with the signals of nearby signalers. By avoiding interference from other signals, non-overlapping signalers are more conspicuous and are thus expected to attract more mates (Greenfield, 1994). When competing signalers mutually offset the timing of their signals in this way, it results in a pattern of signal alternation. This antiphonal signal timing strategy has been broadly observed within acoustically sensitive taxa, including birds (Farabaugh, 1982), insects and anurans (Gerhardt and Huber, 2002).

Alternation of signals in aggregations of animals is widespread. There is, however, considerable variation in signal timing strategies across chorusing species (Wells and Schwartz, 2007). As an extreme opposite to alternation, males of some species produce calls in synchrony, where two or more individuals signal at the same time, maximizing the degree of signal overlap. In anurans, for example, synchronized signaling has been described in a handful of species (Smilisca sila: Tuttle and Ryan, 1982; Dendropsophus ebraccata: Wells and Schwartz, 1984; Cochranella granulosa: Ibáñez, 1993; Kassina fusca: Grafe, 1999; Kassinia kuvage-nus: Grafe, 2003; Hyla arenicolor: unpublished data reviewed in Gerhardt and Huber, 2002; Assa darlingtoni: Clulow et al., 2017; Diasporus diastema: Capshaw et al., 2018). In contrast to the function of signal alternation, increased mate attraction, the function of signal synchrony in these species is less understood (Greenfield, 1994; Gerhardt and Huber, 2002). Prior studies, however, have only examined species that exclusively use one signal timing strategy.

Here we report on the call timing strategies of the Ryukyu Kajika frog (Buergeria japon-ica), a species complex ranging from Taiwan through the Ryukyu Archipelago of Japan. In this species group, specific call properties vary between populations in the region, from island to island (Wang et al., 2017). In most cases, however, calls are generally divided into two distinct types. The first call type (Type I) is a trill that builds in intensity and frequency, from 1.8 kHz to 3 kHz, over its’ duration (Fig. 1a). The second call type (Type II) is a higher dominant frequency (3 kHz) trill of constant intensity, comprised of notes similar to the high frequency notes found at the end of Type I calls (Fig. 1b). Duration is highly variable for both call types, ranging from short bursts, <0.5 s, to prolonged trills, >3.0 s (Kuramoto, 1986). Most notably, field observations suggested that while Type I calls are produced in alternation with neighboring males, Type II calls are produced in synchrony. We followed on these observations by using acoustic playbacks of calls to further determine the timing of the two call types and assess the degree of overlap between synchronized Type II calls. Ultimately, we discuss the selective pressures that might have driven this divergence in timing strategy between Type I and Type II calls in B. japonica.

Materials and Methods

All observational and experimental data was collected from 7 July to 26 July 2015 at B. japonica choruses on Iriomote Island, Japan (24°23'30.3"N, 123°52'48.8"E), located approximately 200 km east of Taiwan in southern Okinawa. Chorus wide call effort (calls/min) of synchronized Type II calls was measured over 10 min intervals (n=11 nights). Acoustic playback experiments were used to assess male B. japonica response latencies to Type I and Type II calls. Playbacks were broadcast using a Pignose portable amplifier speaker (Model 7-100) placed 1 m from a focal male frog in a chorus, at an amplitude of 80 dB SPL re. 20 μP (Brüel and Kjær digital sound level meter Type 2250) at 1 m from the speaker at ground level. Starting 30 min after sunset, a single call, either Type I
or II, was broadcast to the focal frog. Only a single call was broadcast to the focal male and his first vocal response was recorded to assess call timing in response to each call type. Cases in which the focal male responded to the calls of neighboring frogs instead of the playbacks were not included in our analyses. All playbacks were haphazardly drawn from a library of 10 pre-recorded natural calls of each type, each recorded from different individual males. For the duration of this study, temperature ranged from 26.6–29.5°C, relative humidity from 82.4–97.4%, and wind speed from 0.0–4.5 m/s.

For Type I calls, male vocal behaviour was recorded using a Marantz Professional digital recorder (Model PMD660) and a Sennheiser microphone (Model ME66-K6) also placed 1 m from the focal frog (n=27) at ground level next to the speaker. These acoustic recordings were analyzed using CoolEdit2000 (Syntrillium Software). In contrast, for playbacks of Type II calls, timing of vocal responses could not be analyzed acoustically given the high degree of overlap and interference between the playback and the call produced by the focal male and neighboring conspecifics. Instead, we used a sound visualization method (Mizumoto et al., 2011). Specifically, we placed a small sound-to-light device next to a focal male frog in the chorus (n=14 focal males). A second sound-to-light device was placed next to the Pignose speaker 1 m from the frog. The sound-to-light devices contain a microphone and a light emitting diode (LED) which is activated by sound, thus the illumination visually represented sound production. The illumination of both devices, one stimulated by the playback and one stimulated by the frog’s vocal response, was video-recorded (Sony HDR-XR550V, 59.94 fps). The videos were analyzed in MATLAB, following procedures established in Mizumoto et al. (2011). Given the number of frames-per-second at which the videos were recorded, the start of any sound indicated by the sound-to-light devices could be measured at a 16.68 ms resolution. Response time and degree of overlap between the acoustic playback and the focal male’s call were measured and compared.

**Results and Discussion**

Male *B. japonica* responded to playbacks of each call type suggesting that, as in many anurans, this experimental paradigm is an effective method to address vocal competition between males. For playbacks of Type I calls males responded with Type I calls, producing them at a relative delay to the playbacks, avoiding overlap and suggesting an alternating strategy, antiphonal calling. In general,
males called an average of 1.14±0.22 s (±standard error) after the playback had ended (Fig. 2a), never overlapping with the playback. Similarly, male *B. japonica* responded to playbacks of Type II calls with Type II calls, but in this case, focal males synchronized their calls with the playbacks. Males produced Type II calls with an average latency of 0.35±0.05 s, overlapping with the playback calls an average of 72.49±3.13% (Fig. 2b). Thus, the playback experiments showed that males readily offset Type I calls and synchronize Type II calls with neighboring conspecifics to a high degree.

Within anuran species, the immediate timing of calls between males can be fluid, changing based on the acoustic environment or male-male interactions (Wells and Schwartz, 2007). In general, however, for a given species, call timing usually falls into a general pattern of either signal alternation or signal synchronization in which there is overlap between the calls or notes within calls (Wells and Schwartz, 2007). Male *B. japonica* provide a unique example of displaying two distinctly different call timing strategies, antiphonal alternating calls and near-complete overlapped synchronized calls. Although the specific functions of these timing strategies are currently unknown for *B. japonica*, there are several non-mutually exclusive selective pressures that might have driven this divergence between the call types.

In high attendance choruses, numerous males calling in a bout produce an intense cacophony of chorus noise which females can use as a cue to detect and localize the chorus (Wells, 1977). This benefit is reduced when there are fewer males in the aggregation to produce such chorus sound to attract females. Previous studies on *B. japonica*, for example, found that for every 10 additional males in the chorus, the sound pressure level increased by about 3 dB SPL re. 20 μP at 1 m (decibel weighting not reported; Tang, 2009). Males in smaller choruses are thus expected to benefit from synchronized calling by increasing the peak amplitude of their combined calls through constructive interference. This “beacon effect” from synchronized calling has been previously studied in insect choruses (Shelly and Greenfield, 1991; Greenfield, 1994), and might allow male frogs in small choruses to increase the active space of their calls comparable to larger choruses. The benefits of synchrony may thus outweigh the costs when the chorus is small by increasing mate attraction to the chorus but reducing attraction to the individual. When the chorus is large, however, the sound of the chorus already serves as a “beacon”. We observed that *B. japonica* chorus size was highly variable from night to night over the period of this study, reaching over 200 calling males on nights following heavy rains (the largest observed chorus contained 355 males) and dropping below 20 males following multiple days without rain. While it has been reported that *B. japonica* calling effort generally decreases as chorus size decreases (Tang, 2009), how the relative use of each call type changes with chorus size is currently unknown. Furthermore, the assumption for this “beacon” hypothesis is that both call
types function to attracting mates. While there is evidence that both calls play a role in mate attraction (Tang, 2009; Wang et al., 2017), their exact social functions are also unknown. Empirical studies on *B. japonica* are needed to assess call type function and investigate the tradeoff between call alternation and synchrony in regards to chorus size in mate attraction.

In addition to tradeoffs in mate attraction, call timing may also be driven by nontarget receivers of mating signals, eavesdropping predators (Ryan et al., 1982). Calling *B. japonica* attract multiple species of frog-biting midges (*Corethrella* spp.) and mosquitoes (*Uranotaenia* spp.), which use frog calls as cues to localize male frogs and take blood meals (Toma et al., 2014). Frog-biting insects can impose costs on male frogs in the form of blood loss (Camp, 2006) and parasite infection (Johnson et al., 1993; Bernal and Pinto, 2016). Male *B. japonica* may synchronize their calls to reduce the ability of predators to localize individual signals, acoustically masking their calls with those of neighboring males (the eavesdropper avoidance hypothesis: Tuttle and Ryan, 1982). How call synchrony can reduce eavesdropper attraction while not also reducing female attraction is being currently investigated in other anuran species (Legett et al., 2019).

Finally, we observed that synchronized *B. japonica* Type II calls were produced sporadically and at low rates (0.61 ±0.19 calls/min). This calling pattern is characteristic of choruses of other synchronizing species such as the neotropical pug-nosed treefrog (*S. sila*: Ryan, 1986) and the Kuvangu African running frog (*K. kuvangensis*: Grafe, 2003) suggesting similar selective pressures may have driven convergent evolution of calling strategies in these distantly related species. Overall, by displaying two distinct call timing strategies, *B. japonica* provide an ideal model for studying the costs and benefits of both call alternation and synchronization in anurans. Future studies investigating this species would provide insights into the function of signal timing in anurans and, more broadly, how mating signals are shaped by social and environmental pressures.

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