

**THE FUNCTION OF FINE-SCALE SIGNAL TIMING STRATEGIES:
SYNCHRONIZED CALLING IN STREAM BREEDING TREE FROGS**

by

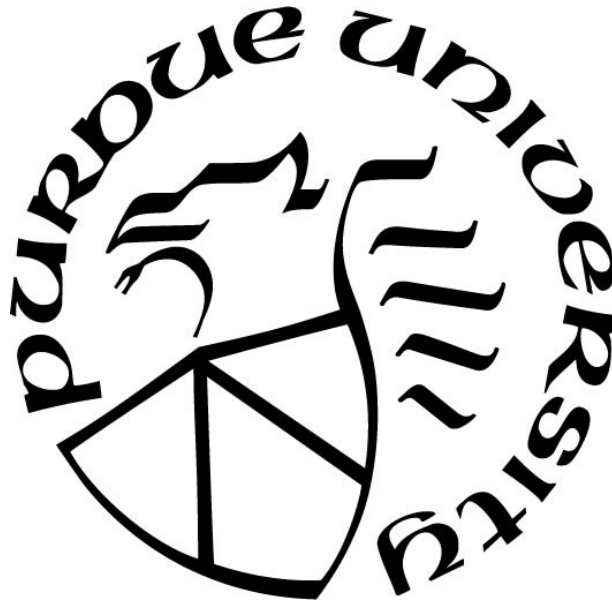
Henry Daniel Legett

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy



Department of Biological Sciences

West Lafayette, Indiana

May 2020

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Jeffrey R. Lucas, Chair

Department of Biological Sciences

Dr. Ximena E. Bernal

Department of Biological Sciences

Dr. Esteban Fernández-Juricic

Department of Biological Sciences

Dr. Jason T. Hoverman

Department of Forestry and Natural Resources

Approved by:

Dr. Janice P. Evans

Head of the Departmental Graduate Program

Dedicated to Mom, Dad, Rebecca, and Sam

ACKNOWLEDGMENTS

The research in this dissertation was funded by the National Science Foundation (IOS no. 1433990 to X.E. Bernal), the Japan Society for the Promotion of Science (NSF EAPSI no. 1515380 to H. D. Legett), an A. Stanley Rand Fellowship from the Smithsonian Tropical Research Institute, an A. A. Lindsey Graduate Fellowship and a Graduate School Summer Research Grant from Purdue University, and a student research grant from the Animal Behavior Society. The writing of this dissertation was additionally supported by a Bilsland Dissertation Fellowship from Purdue University. I thank the Smithsonian Tropical Research Institute (STRI) and the Tropical Biosphere Research Center of the University of the Ryukyus for logistical support, as well as Ryan Taylor and Kimberly Hunter for the use of their anechoic sound chamber in Gamboa. I also thank the faculty and staff at Purdue's Department of Biological Sciences, especially the business office, Gina Rupp, Denise Zielinski, and Kerry Rabenold for their help throughout my time at Purdue.

I am thankful for the support and friendship of many incredible graduate students, especially my teammates in the Bernal Lab: Sara Zlotnik, Stephanie Gutierrez, and Brian Leavell. I am grateful to Luke Tyrrell and Alyssa Gleichsner for mentoring me in my early years as a graduate student. I never would have made it past the first year without them. I additionally thank Nigel Anderson, Samuel Freedlund, Grace Higginbottom, Tsun Lai 'Alfa' Lam, Suzette Miller, and Carlos Pantoja for their help analyzing hundreds of hours of video and audio recordings, and Ryan Madden for help with field and laboratory work.

I thank my collaborators, Rachel Page and Ikkyu Aihara, for their hospitality and advice during my travels. I thank the members of my committee, Esteban Fernández-Juricic and Jason Hoverman, for their suggestions and feedback. I am especially grateful to Jeff Lucas for being a role model of scientific merit, and whose uncompromising criticism and support are the foundations of this dissertation. Finally, I consider myself absolutely blessed to have been advised by Ximena Bernal. It was only through her mentorship and patience that I am the passable scientist that I am today. The research presented here is directly owed to her knowledge and effort.

TABLE OF CONTENTS

LIST OF TABLES.....	8
LIST OF FIGURES	9
ABSTRACT.....	11
INTRODUCTION	12
CHAPTER 1. SYNCHRONIZED MATING SIGNALS IN A COMMUNICATION NETWORK: THE CHALLENGE OF AVOIDING PREDATORS WHILE ATTRACTING....	17
1.1 Abstract.....	17
1.2 Introduction.....	18
1.3 Methods.....	21
1.3.1 Risk of predation by frog-eating bats	21
1.3.2 Risk of attack by frog-biting midges	23
1.3.3 Attractiveness to female frogs	23
1.3.4 Statistical analysis.....	24
1.4 Results.....	25
1.4.1 Risk of predation by frog-eating bats	25
1.4.2 Risk of attack by frog-biting midges	26
1.4.3 Attractiveness to female frogs	26
1.5 Discussion.....	27
1.5.1 Signal synchronization in the context of multiple eavesdroppers	27
1.5.2 Implications for the origin and maintenance of signal synchronization.....	28
1.5.3 Other functions of signal synchronization in anurans and other taxa.....	30
1.6 Conclusion	32
1.7 References.....	40
CHAPTER 2. PREY EXPLOITS THE AUDITORY ILLUSIONS OF EAVESDROPPING PREDATORS	45
2.1 Abstract.....	45
2.2 Introduction.....	46
2.3 Methods.....	47
2.3.1 Frog-eating bat preference.....	48

2.3.2	Frog-biting midge preference	49
2.3.3	Female frog preference	49
2.3.4	Statistical analyses	50
2.4	Results.....	50
2.5	Discussion.....	51
2.6	References.....	56
CHAPTER 3. SIGNAL SYNCHRONY AND ALTERNATION AMOUNG NEIGHBORING MALES IN A JAPANESE STREAM BREEDING TREEFROG, <i>BUERGERIA JAPONICA</i>		59
3.1	Abstract.....	59
3.2	Introduction.....	60
3.3	Methods.....	61
3.4	Results and Discussion	62
	65	
3.5	References.....	67
CHAPTER 4. WITHIN HOST ACOUSTIC SIGNAL PREFERENCE OF FROG-BITING MOSQUITOES (DIPTERA: CULICIDAE) AND MIDGES (DIPTERA: CORETHRELLIDAE) ON IRIOMOTE ISLAND, JAPAN		69
4.1	Abstract.....	69
4.2	Introduction.....	70
4.3	Methods.....	71
4.3.1	Eavesdropper selection for call type.....	71
4.3.2	Statistical Analysis.....	72
4.4	Results.....	73
4.5	Discussion.....	73
4.6	References.....	79
CHAPTER 5. THE DUAL BENEFITS OF SYNCHRONIZED MATING SIGNALS IN A JAPANESE TREEFROG: ATTRACTING MATES AND MANIPULATING PREDATORS .		82
5.1	Abstract.....	82
5.2	Introduction.....	83
5.3	Methods.....	85
5.3.1	Study system and location	85

5.3.2	Acoustic playbacks	85
5.3.3	Signal transmission.....	86
5.3.4	Female synchronized-unsynchronized call preference	86
5.3.5	Leading-following call preferences in eavesdropping predators	87
5.3.6	Leading-following call preferences in female frogs	87
5.3.7	Statistical analyses	88
5.4	Results.....	89
5.4.1	Signal transmission.....	89
5.4.2	Female synchronized-unsynchronized call preference	89
5.4.3	Leading-following call preferences in eavesdropping predators	89
5.4.4	Leading-following call preferences in female frogs	89
5.5	Discussion	90
5.5.1	Signal synchronization as a ‘beacon’ for females	90
5.5.2	Synchronization as a predator avoidance strategy	92
5.5.3	Relaxed sexual selection for synchronization.....	93
5.5.4	Synchronization in anurans compared to insects and other tax	94
5.6	References.....	103
CONCLUSION		108

LIST OF TABLES

Table 1.1. Tukey comparisons for bat attacks on pug-nosed tree frog (a) and túngara frog (b) calls, and midges attracted to pug-nosed tree frog (c) and túngara frog (d) calls. Table corresponds to Figure 1.4. The values for least squares means estimate, standard error, t -ratio, and p -value were calculated using the *emmeans* R package. Cohn’s d effect size was calculated using the *lsr* R package. 33

Table 4.1. Numbers of insects collected by frog call traps broadcasting either Type I or Type II Ryukyu Kajika frog calls. Mosquitoes (Culicidae) and midges (Corethrellidae) were analyzed using GLMMs with zero-inflated Poisson error structures, moth flies (Psychodidae) were analyzed using a GLMM with a non-zero-inflated error structure, and non-eavesdropping insects (Bycatch) were analyzed using a GLMM with a negative binomial II error structure ($n = 30$ nights, $df = 53$). Statistically significant differences ($p < 0.05$) are indicated with a “*”. 76

Table 5.1. Natural *B. japonica* call characteristics (dominant frequency and call duration) from previous studies compared to the characteristics of the synthetic call used in this study..... 96

Table 5.2. Differences in signal attenuation (a) and spectral degradation (b) between broadcast synchronized and unsynchronized *B. japonica* calls rerecorded at distances of 1 – 80 m. Values are the average across five inner forest sites (mean $\pm$ se). Attenuation compares the amplitude (dB) of the dominant frequency of the calls, while degradation compares Pearson correlation coefficients. 97

LIST OF FIGURES

Figure 1.1. Experimental set-ups for the receiver phonotaxis experiments. For the eavesdropper, nontarget receiver experiments (a), three stations of two speakers were positioned 10 m apart. Each of the three speaker stations simultaneously broadcast one of the three treatments: (i) calls at near-perfect synchrony (5 ms of latency), (ii) calls at natural average synchrony (79 ms of latency), or (iii) calls out of synchrony (alternating calls). Eavesdropping midge and bat attraction to the focal speaker (the speaker broadcasting “following” calls) was compared across treatments. For the target receiver experiment (b), two speaker stations were positioned 3 m apart in a 2 m×3 m semi-anechoic chamber. Each station broadcast either (i) synchronized calls (79 ms of latency) or (ii) alternating calls. The near-perfect synchrony treatment (5 ms latency) was not included in the target receiver experiment because the semi-anechoic sound chamber was restricted to 4 speakers (2 speaker station choice tests). Of the two synchrony treatments, calls broadcast with the average latency (79 ms) was chosen. 34

Figure 1.2. Oscillogram (top) and spectrogram (bottom) of the mating calls of the pug-nosed tree frog (a) and túngara frog (b). The oscillogram shows two calls synchronized with a latency of 79 ms, the average natural synchrony of pug-nosed tree frogs. The spectrogram shows only the first call. Although both pug-nosed tree frogs and túngara frogs can produce a more complex, multi-note call, only playbacks of simple calls (as shown) were used in this study. 35

Figure 1.3. Method for creating stimuli, treatments (5 ms, 79 ms, alternating calls) for each trial. Pre-recorded calls were randomly drawn from the library of 10 (either *Smilisca sila* or *Engystomops pustulosus*) so that the same call was not repeated within a single trial. Specific call combinations were not repeated for a receiver (bat, midge, or female frog). Note that as a result of leader-follower preferences [1], the order in which calls are presented in the 5 ms and 79 ms treatments change the way these stimuli are perceived (e.g. call #8 and call #2 broadcast at 5 ms overlap is different than call #2 and call #8 broadcast at 5 ms overlap). Thus, from the library of 10 calls, there were 90 potential stimuli each for the 5 ms and 79 ms treatments, and 45 combinations for the alternative treatment. 36

Figure 1.4. Eavesdropper preferences for synchronized and unsynchronized calls, measured for both pug-nosed tree frog (left) and túngara frog (right) calls during each species’ respective breeding season. Number of bat attacks per night (a,b) and number of midges captured per night (c,d) for calls were compared for different call timings: near-perfect synchrony (5 ms of latency), average pug-nosed tree frog call synchrony (79 ms of latency) and alternating calls (Alt). Values are the least squared means and bars show standard error. Note that for midge attraction, the y-axis range for pug-nosed tree frogs (c) is different than túngara frogs (d). 37

Figure 1.5. Female preference for synchronized and unsynchronized calls, measured for both pug-nosed tree frog (a) and túngara frog (b) calls during each species’ respective breeding season. Female choice was compared between calls broadcast in synchrony with 79 ms of latency between calls (Synch), or broadcast in alternation (Alt). Each choice represents a single female. 38

Figure 1.6. Phylogeny of *Smilisca* clade (Smith et al. 2007). Circles following each species name show call timing strategy, assigned based on literature (Savage 2002) and from call recordings. While *S. sordida*, the sister species of *S. sila*, is described as having alternating calls, male *S.*

sordida often call in a rapid sequential “domino” pattern (p. 357 in Savage 2002) that can result in signal overlap. Open circles indicate non-synchronous calling, black circles indicate the use of synchronized calls, and gray circles indicate an intermediate calling strategy: alternating calls with some overlap. 39

Figure 2.1. Oscillograms (a) of leading and following calls of two male pug-nosed treefrogs, and (b) the target and non-target call receivers included in this study. The calls are synchronized with 79 ms of latency, the observed natural average in this species (Ryan 1986). 54

Figure 2.2. The proportion of non-target (a bats and b midges) and target (c female pug-nosed frogs) receivers that selected for leading or following recorded calls of male pug-nosed treefrogs. The preferences of another target receiver, female túngara frogs (d), were assessed using male túngara frog calls. 55

Figure 3.1. Oscillograms and spectrograms of Type I (a) and Type II (b) mating calls. 65

Figure 3.2. Average timing of male responses to Type I (a) and Type II (b) call playbacks. Bars show standard error. 66

Figure 4.1. Oscillogram (top) and spectrogram (bottom) of a Type I (a) and Type II (b) Ryukyu Kajika frog call. 77

Figure 4.2. Frog-biting mosquitoes of the genus *Uranotaenia* (a), frog-biting midges of the genus *Corethrella* (b), moth flies (c), and bycatch (d) attracted to either Type I or Type II Ryukyu Kajika frog calls. Values are means and standard errors. Statistically significant differences between call types ($p < 0.05$) are indicated with a “*”. Note the difference in scale for bycatch. 78

Figure 5.1. The study system (a), including the sender, target receiver, and nontarget receivers of *B. japonica* calls. Oscillograms and spectrograms (b) of a natural *B. japonica* call and the synthetic call used in this study. 98

Figure 5.2. Attenuation (a) and degradation (b) of broadcast synchronized and unsynchronized *B. japonica* calls. Values for attenuation are the amplitude (dB) of the dominant frequency (3.25 kHz), while values for degradation are Pearson correlation coefficients. 99

Figure 5.3. Circular diagram depicting the angle (to the nearest 15° increment) at which female *B. japonica* left the phonotaxis arena with synchronized calls broadcast at 0° and unsynchronized calls broadcast at 180°. Both stimuli were located at a distance 40 m from the center of the arena. Each circle represents an individual female frog. 100

Figure 5.4. Eavesdropper preferences for leading or following calls. Numbers of midges (a) and mosquitoes (b) are the average collected per night (mean ± se). 101

Figure 5.5. Female *B. japonica* preference for leading or following calls. Each female choice represents a single female. 102

ABSTRACT

In dense mating aggregations, such as insect and anuran choruses, signals produced at the same time can overlap and interfere with one another, reducing the ability of receivers to discriminate between individual signals. Thus, evolution by sexual selection is expected to result in mating signal timing strategies that avoid overlap. Patterns of signal alternation between competing males are commonly observed in leks and choruses across taxa. In some species, however, signalers instead deliberately overlap, or ‘synchronize’, their mating signals with neighboring conspecifics. Given the assumed high cost of reduced mate attraction when signals overlap, mating signal synchronization has remained an evolutionary puzzle. Synchronization may be beneficial, however, if overlapping signals reduce the attraction of nontarget receivers (predator avoidance hypothesis). Synchronized signals could also constructively interfere, increasing female attraction to the mating aggregation (the beacon effect hypothesis). I investigate these functions of synchronized signaling in two species of tree frogs that synchronize their mating calls: the pug-nosed tree frog (*Smilisca sila*) and the Ryukyu Kajika frog (*Buergeria japonica*). To examine the trade-offs imposed by call synchronization in each species, I conduct a series of field and laboratory playback experiments on target (female frogs) and nontarget (eavesdropping predators) receivers of frog calls. Results from these experiments support both hypotheses, suggesting that synchronized frog calls can reduce the attraction of predators and attract mates to the chorus. In addition, I found reduced preferences for fine-scale call timings in female *S. sila* and *B. japonica*, deviating from the expected preferences observed in many other anuran and non-anuran species. Thus, while males may enjoy multiple benefits from synchronized mating signals, relaxed sexual selection for non-synchronous signals may be key to the evolution and maintenance of mating signal synchrony.

INTRODUCTION

Males of many species aggregate and produce conspicuous displays to attract mates. Notable examples of this phenomenon include the choruses and leks of birds, insects, and anurans.

Attracting mates in aggregations can be challenging, however, as mating signals produced at the same time by neighboring conspecifics can overlap. Overlapped mating signals have two main disadvantages compared to non-overlapped signals. First, signals can interfere, reducing the ability of females to discriminate between individual signals within aggregations (Wollerman and Wiley 2002a; Wollerman and Wiley 2002b). Second, when signals overlap but are offset, females of many species prefer the initial ‘leading’ signal to the lagging ‘following’ signal, a phenomenon known as the ‘precedence effect’ or ‘leader-follower preferences’ (Klump and Gerhardt 1992; Greenfield et al. 1997). For these reasons, competing males in aggregations often use fine-scale signal timing strategies to avoid overlap, offsetting the timing of signal production in an alternating pattern. Signal alternation is commonly observed in aggregations across taxa (birds: Farabaugh 1982; insects and anurans: Gerhardt and Huber 2002) and signal modalities (acoustic and visual: Carlson and Copeland 1985; Morin 1986).

Alternation of signals is widespread. There is, however, considerable variation in signal timing strategies across species (Wells and Schwartz 2007). Males of some species even produce calls in ‘synchrony’, deliberately overlapping their mating signals with those produced by neighboring conspecifics in a chorus (insects and anurans: Greenfield 1994a; birds: Hall 2009; mammals: Harrington and Mech, 1979; crustaceans: Reaney et al. 2008). Unlike the advantages of signal alternation, increased female attraction to the individual, the advantages of mating signal synchronization have remained a mystery. Furthermore, while multiple hypotheses have been proposed to explain synchronous strategies, empirical considerations of the subject are lacking.

Typically, signal synchrony has been explained as an outcome of male-male competition, in which a male tries to mask a neighbor’s signal with his own signal (Wells 1977; Greenfield 1994b). However, while the neighboring male’s attractiveness may be reduced, the synchronizing male also masks his own signal reducing his own attractiveness. Thus, this function of mating signal synchrony seems unlikely (Well 1977). If males rhythmically signal at

regular time intervals, as observed in many cricket and katydid species, males might be able to predict the timing of a competitor's signal and place his own signal in the more attractive leading position (Greenfield et al. 1997). In many synchronizing anuran and insect species, however, males actively place their signals in following positions in response to neighboring males (Ryan 1986; Grafe 1999; Hartbauer et al. 2005).

While females impose strong selective pressure on male displaying strategies, signaling also exposes males to eavesdropping predators and parasites (Zuk and Kolluru 1998). Just as signal overlap may reduce female attraction, so to might overlap reduce the attraction of nontarget receivers of mating signals. As a result, predator avoidance is commonly proposed as a possible function of synchronous signaling (the 'eavesdropper avoidance' hypothesis: Tuttle and Ryan 1982; Greenfield 1994a; Gerhardt and Huber 2002; Wells and Schwartz 2007). Despite the popularity of this explanation, only one study has examined how the fine-scale timing of signals between males affects predator attraction (Tuttle and Ryan 1982). In addition to the thin evidence supporting this hypothesis, synchronized signaling strategies are also observed in systems with few eavesdropping predators, such as on islands (e.g. Legett et al. in prep.).

Overlapping signals may also benefit males if they constructively interfere, increasing peak signal amplitude (the 'beacon effect' hypothesis: Buck and Buck 1978). Increasing signal amplitude could increase female attraction in multiple, related ways. In general, females prefer signals of higher intensity (Ryan and Keddy-Hector 1992; Gerhardt 1987), using call intensity to select for larger, more preferred males (Gerhardt 1975). Overlapping signals of higher amplitude could also make a chorus more detectable from a greater distance (Gerhardt 1975; Penna and Solis 1998; Gerhardt and Klump 1988). Thus, increasing the active space of the overlapping signals and the range over which females are attracted (Wells 1977; Gerhardt and Klump 1988). Previously, this benefit of signal overlap has received some attention in relation to group size and the evolution of aggregate breeding (Ryan et al. 1981; Kokko et al. 1998). In acoustically signaling insects, for example, the overlapping songs of multiple male crickets in a chorus have a higher peak amplitude than single males, and the number of females approaching per male is higher for aggregated signalers (Shelly and Greenfield 1991). Similarly, in anurans, aggregated callers produce higher amplitude 'chorus noise' which is used by both male and female anurans

to locate choruses of conspecifics (Bee 2007; Buxton et al. 2015). Thus, for the beacon effect, the benefits of signal synchronization are analogous to the benefits males enjoy by aggregating in general. At this time, however, studies have focused only on the number of males in a chorus, not the effects of fine-scale timing between signals.

Both the eavesdropper avoidance and beacon effect hypotheses of mating signal synchronization ignore the potential cost of reduced attractiveness to females. Female choice is assumed to prevent the use of synchronized signals, given that females prefer non-overlapping signals (Schwartz 1987; Bee and Micheyl 2008) and are considered the primary driver of mating signal evolution (Kirkpatrick and Ryan 1991; Andersson 1994). For the eavesdropper avoidance hypothesis, male signalers must balance the attraction of predators and parasites against the attraction of females. For the beacon effect hypothesis, male signalers must balance female attraction to the aggregation against female attraction to signals produced by individual males once females are within the aggregation. Yet, despite the attention that both hypotheses have previously received, these tradeoffs have remained unexplored.

The following dissertation focuses on the eavesdropper avoidance and beacon effect hypotheses of mating signal synchronization, specifically considering the tradeoffs between the benefits of signal synchrony and the costs to female attraction. In Chapter 1, I examine the attraction of eavesdropping predators to synchronized and unsynchronized signals in two Neotropical anuran species: pug-nosed tree frogs (*Smilisca sila*), in which males naturally produce mating calls in synchrony, and túngara frogs (*Engystomops pustulosus*), in which males naturally alternate their calls. I also assess differences in female attraction to the synchronized and unsynchronized calls of both anuran species. In Chapter 2, I examine the leader-follower preferences of eavesdropping predators and female frogs to synchronized *S. sila* calls. In Chapter 3, I describe the signal timing strategies of a Japanese stream breeding treefrog, the Ryukyu Kajika frog (*Buergeria japonica*). In Chapter 4, I examine the eavesdropper community that preys on calling male *B. japonica* and assess eavesdropper attraction to the different call types of this species. In Chapter 5, I evaluate two components of the beacon effect hypothesis: how synchronized and unsynchronized *B. japonica* calls transmit through the environment, and female *B. japonica* attraction to synchronized and unsynchronized calls from a distance beyond the edge of the chorus. Finally, I

also assess the leader-follower preferences of eavesdropping predators and female frogs to synchronized *B. japonica* calls.

References

- Andersson, M. B. (1994). *Sexual selection*. Princeton University Press, Princeton.
- Bee, M. A. (2007). Selective phonotaxis by male wood frogs (*Rana sylvatica*) to the sound of a chorus. *Behavioral Ecology and Sociobiology* 61: 955-966.
- Bee, M. A., and Micheyl, C. (2008). The cocktail party problem: what is it? How can it be solved? And why should animal behaviorists study it?. *Journal of Comparative Psychology* 122: 235-251.
- Buck, J., and Buck, E. (1978). Toward a functional interpretation of synchronous flashing by fireflies. *The American Naturalist* 112: 471-492.
- Buxton, V. L., Ward, M. P., and Sperry, J. H. (2015). Use of chorus sounds for location of breeding habitat in 2 species of anuran amphibians. *Behavioral Ecology* 26: 1111-1118.
- Carlson, A. D., and Copeland, J. (1985). Flash communication in fireflies. *The Quarterly review of biology* 60: 415-436.
- Farabaugh, S. M. (1982). The ecological and social significance of duetting. In *Acoustic communication in birds, Vol. 2 Song learning and its consequences*, (eds. D. E. Kroodsma and E. H. Miller) pp. 85-124. Academic Press, New York.
- Gerhardt H.C., and Klump G.M. (1988). Masking of acoustic signals by the chorus background noise in the green tree frog: A limitation on mate choice. *Animal Behaviour* 36: 1247-1249.
- Gerhardt, H. C. (1975). Sound pressure levels and radiation patterns of the vocalizations of some North American frogs and toads. *Journal of Comparative Physiology* 102: 1-12.
- Gerhardt, H. C. (1987). Evolutionary and neurobiological implications of selective phonotaxis in the green treefrog, *Hyla cinerea*. *Animal Behaviour* 35: 1479-1489.
- Gerhardt, H. C., and Huber, F. (2002). *Acoustic communication in insects and anurans: common problems and diverse solutions*. University of Chicago Press, Chicago.
- Grafe, T. U. (1999). A function of synchronous calling and a novel female preference shift in an anuran. *Proceedings of the Royal Society B* 266: 2331-2336.
- Greenfield, M. D. (1994a). Synchronous and alternating choruses in insects and anurans: common mechanisms and diverse functions. *American Zoologist* 34: 605-615.
- Greenfield, M. D. (1994b). Cooperation and conflict in the evolution of signal interactions. *Annual Review of Ecology and Systematics* 25: 97-126.
- Greenfield, M. D., Tourtellot, M. K., and Snedden, W. A. (1997). Precedence effects and the evolution of chorusing. *Proceedings of the Royal Society B* 264: 1355-1361.
- Hall, M. L. (2009). A review of vocal duetting in birds. *Advances in the Study of Behavior* 40: 67-121.
- Harrington, F. H., and Mech, L. D. (1979). Wolf howling and its role in territory maintenance. *Behaviour* 68: 207-249.
- Hartbauer, M., Kratzer, S., Steiner, K., and Römer, H. (2005). Mechanisms for synchrony and alternation in song interactions of the bushcricket *Mecopoda elongata* (Tettigoniidae: Orthoptera). *Journal of Comparative Physiology A* 191: 175-188.

- Kirkpatrick, M., and Ryan, M. J. (1991). The evolution of mating preferences and the paradox of the lek. *Nature* 350: 33-38.
- Klump, G. M., and Gerhardt, H. C. (1992). Mechanisms and function of call-timing in male-male interactions in frogs. In *Playback and studies of animal communication*. (ed. P. K. McGregor), pp. 153–174. Plenum Press, New York.
- Kokko, H., Sutherland, W. J., Lindstrom, J., Reynolds, J. D., and Mackenzie, A. (1998). Individual mating success, lek stability, and the neglected limitations of statistical power. *Animal Behaviour* 56: 755-762.
- Legett, H. D., Aihara, I., and Bernal, X. E. (in prep.). Within host acoustic signal preference of frog-biting mosquitoes (Diptera: Culicidae) and midges (Diptera: Corethrellidae) on Iriomote Island, Japan.
- Morin, J. G. (1986). Firefleas of the sea: luminescent signaling in marine ostracode crustaceans. *Florida Entomologist* 69: 105-121.
- Penna, M., and Solís, R. (1998). Frog call intensities and sound propagation in the South American temperate forest region. *Behavioral Ecology and Sociobiology* 42: 371-381.
- Reaney, L. T., Sims, R. A., Sims, S. W., Jennions, M. D., and Backwell, P. R. (2008). Experiments with robots explain synchronized courtship in fiddler crabs. *Current Biology* 18: R62-R63.
- Ryan, M. J. (1986). Synchronized calling in a treefrog (*Smilisca sila*). *Brain, Behavior and Evolution* 29: 196-206.
- Ryan, M. J., and Keddy-Hector, A. (1992). Directional patterns of female mate choice and the role of sensory biases. *The American Naturalist* 139: S4-S35.
- Ryan, M. J., Tuttle, M. D., and Taft, L. K. (1981). The costs and benefits of frog chorusing behavior. *Behavioral Ecology and Sociobiology* 8: 273-278.
- Schwartz, J. J. (1987). The function of call alternation in anuran amphibians: a test of three hypotheses. *Evolution* 41: 461-471.
- Shelly, T. E., and Greenfield, M. D. (1991). Dominions and desert clickers (Orthoptera: Acrididae): influences of resources and male signaling on female settlement patterns. *Behavioral Ecology and Sociobiology* 28: 133-140.
- Tuttle, M. D., and Ryan, M. J. (1982). The role of synchronized calling, ambient light, and ambient noise, in anti-bat-predator behavior of a treefrog. *Behavioral Ecology and Sociobiology* 11: 125-131.
- Wells, K. D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour* 25: 666-693.
- Wells, K. D., and Schwartz, J. J. (2007). The behavioral ecology of anuran communication. pp. 44-86. In: *Hearing and sound communication in amphibians*. Springer, New York.
- Wollerman, L., and Wiley, H. R. (2002a). Possibilities for error during communication by neotropical frogs in a complex acoustic environment. *Behavioral Ecology and Sociobiology* 52: 465-473.
- Wollerman, L., and Wiley, R. H. (2002b). Background noise from a natural chorus alters female discrimination of male calls in a Neotropical frog. *Animal Behaviour* 63: 15-22.
- Zuk, M., and Kolluru, G. R. (1998). Exploitation of sexual signals by predators and parasitoids. *The Quarterly Review of Biology* 73: 415-438.

CHAPTER 1. SYNCHRONIZED MATING SIGNALS IN A COMMUNICATION NETWORK: THE CHALLENGE OF AVOIDING PREDATORS WHILE ATTRACTING

H. D. Legett¹, R. A. Page², X. E. Bernal^{1,2}

¹ *Purdue University, West Lafayette, IN, USA*

² *Smithsonian Tropical Research Institute, Balboa, Ancón, Panama, PA*

Citation

This chapter was originally published in the journal *Proceedings of the Royal Society B* and is reproduced here under the Royal Society standard license to publish.

Legett, H. D., Page, R. A., and Bernal, X. E. (2019). Synchronized mating signals in a communication network: the challenge of avoiding predators while attracting mates. *Proceedings of the Royal Society B*, 286: 20191067.

1.1 Abstract

Conspicuous mating signals attract mates but also expose signalers to predators and parasites. Signal evolution, therefore, is driven by conflicting selective pressures from multiple receivers, both target and nontarget. Synchronization of mating signals, for example, is an evolutionary puzzle given the assumed high cost of reduced female attraction when signals overlap. Synchronization may be beneficial, however, if overlapping signals reduce attraction of nontarget receivers. We investigate how signal synchronization is shaped by the tradeoff between natural and sexual selection in two anuran species: pug-nosed tree frogs (*Smilisca sila*), in which males produce mating calls in near-perfect synchrony, and túngara frogs (*Engystomops pustulosus*), in which males alternate their calls. To examine the tradeoff imposed by signal synchronization, we conducted field and laboratory playback experiments on eavesdropping enemies (bats and midges) and target receivers (female frogs). Our results suggest that, while synchronization can be a general strategy for signalers to reduce their exposure to eavesdroppers, relaxed selection by females for unsynchronized calls is key to the evolution and maintenance of signal synchrony. This study highlights the role of relaxed selection in our understanding the origin of mating signals and displays.

1.2 Introduction

Most communication occurs in a network, where signals are detected and used by multiple receivers, both target and nontarget (Zuk and Kolluru 1998, McGregor and Peake 2000). This is true across scales, systems, and sensory modalities, from the molecules exchanged between bacteria (Taga and Bassler 2003) to the complex multimodal courtship displays exhibited by many birds, fish, mammals, and insects (Hebets and Papaj 2005). Despite the pervasive nature of communicating in networks, communication is most often investigated as if occurring in a signaler-receiver dyad. In that framework, a sender produces a signal that is transmitted through the environment and detected by a single target receiver. Empirical studies on signal evolution, for example, have predominantly focused on the selective pressures imposed by mates (McGregor 2005), given that females are the main target receiver of mating signals. Other receivers have also been considered independently such as competitors (McGregor and Peake 2000) and nontarget receivers such as predatory or parasitic eavesdroppers, the unintended recipients of signals (Zuk and Kolluru 1998, Peake 2005). A dyadic approach, however, ignores the conflicting influence of both target and nontarget receivers on signals, and the signaling tradeoffs that might ensue. In this study, we use a communication network perspective (Peake 2005) that considers multiple target and nontarget receivers to examine signal timing strategies in anuran choruses. We investigate how selective pressure from different receivers has resulted in an unexpected outcome, signal synchronization.

In dense mating aggregations, such as insect and anuran choruses, males of many species use signal timing strategies to avoid signal overlap with neighboring conspecifics (the “cocktail party problem”, Bee and Micheyl 2008). Overlapped mating signals have two main disadvantages compared to non-overlapped signals. For one, signals produced at the same time can interfere, reducing the ability of the females to recognize individual signals (Wollerman 1999) and discriminate between males displaying at aggregations (Wollerman and Wiley 2002a; Wollerman and Wiley 2002b). Additionally, when signals overlap but are offset, females of many species prefer the initial “leading” signal to the second “following” signal, commonly referred to as the “precedence effect” or “leader-follower preferences” (Whitney and Krebs 1975, Klump and Gerhardt 1992, Grafe 1996, Greenfield et al. 1997, Bosch and Márquez 2002, Höbel 2010). For these reasons, competing males often offset the timing of their signals, in a

pattern of signal alternation. An alternation signal timing strategy is commonly observed across taxa (insects and anurans: Gerhardt and Huber 2002; birds: Farabaugh 1982) and signal modalities (acoustic and visual: Carlson and Copeland 1985; Morin 1986). Signals produced in alternation do not interfere with one another and are therefore more conspicuous, increasing the likelihood of female attraction. More conspicuous signals, however, may also increase the likelihood of attracting nontarget eavesdroppers. Inversely, signals that do overlap are less conspicuous, and potentially less attractive to eavesdroppers (Tuttle and Ryan 1982).

Although overlapping signals are not as common as alternating signals, males of some species do deliberately overlap, or “synchronize”, their mating signals with neighboring males (insects and anurans: Greenfield 1994; birds: Hall 2009; mammals: Harrington and Mech 1979; crustaceans: Reaney et al. 2008). A synchronized timing strategy has been proposed to function in evading detection by predatory or parasitic eavesdroppers (Tuttle and Ryan 1982). By effectively masking their own signals with those of neighboring competitors, male signalers can reduce the attraction of unwanted receivers. This hypothesis has rarely been tested, however, and a crucial, unresolved piece of this puzzle is how signal synchronization affects female attraction. Given the selective pressure imposed by both mates and eavesdroppers, a comprehensive understanding of the evolution of mating signals requires considering both types of receivers. Here, we use a communication network approach to examine receiver attraction to synchronized mating signals. In particular, we investigate the costs and benefits imposed by mates and multiple eavesdroppers on signal timing strategies in the pug-nosed tree frog (*Smilisca sila*) and the túngara frog (*Engystomops pustulosus*).

Chorusing male pug-nosed tree frogs synchronize their calls with those of neighboring males at extremely short latencies, with a minimum delay of 5 ms and an average delay of 79 ms (Ryan 1986). While pug-nose tree frogs call during the dry season, in the same habitat, male túngara frogs form dense choruses during the rainy season. Unlike pug-nosed tree frogs, neighboring male túngara frogs alternate the timing of their mating calls (Ryan 1985). Both pug-nosed tree frogs and túngara frogs are preyed upon by frog-eating bats (*Trachops cirrhosis*: Ryan et al. 1982; Tuttle and Ryan 1982) and frog-biting midges (*Corethrella* spp.: Legett et al. 2018). These bats and midges use the mating calls of the frogs as a cue to localize calling males (Page et al.

2014). Attracting bats has a direct fitness cost to a calling male frog: predation. While frog-biting midges only take small blood meals, a single calling male túngara frog can attract hundreds of midges in half an hour (Bernal et al. 2006). For male túngara frogs, such high attack rates can potentially contribute to high costs from blood loss, which may be equivalent to about 10% of their blood volume in a night of calling (unpublished calculations based on estimates of the amount of blood collected by a single fly: Camp 2006). Additionally, frog-biting midges are themselves vectors for blood parasites (Johnsons et al. 1993; Bernal and Pinto 2016). We consider these two anuran species, pug-nosed tree frogs and túngara frogs, that share their main eavesdroppers but have distinctly different call timing strategies. For each species we examine three receivers from the communication network: female frogs, frog-eating bats and frog-biting midges.

We investigate the effectiveness of signal synchronization as a strategy for reducing attraction to multiple eavesdroppers, and address the conundrum of how females select mates in a synchronized chorus. Specifically, we examine the potential costs of reduced attractiveness to females and the benefits of reduced risk of attacks by predators associated with producing calls in synchrony versus alternating calls with those of neighboring males. Typically, female choice is expected to prevent signal synchronization given that females are considered the primary driver of courtship signal evolution (Kirkpatrick and Ryan 1991; Andersson 1994), and they prefer non-overlapping calls to avoid localization and discrimination challenges (Schwartz 1987; Bee and Micheyl 2008). For synchronization to evolve or be maintained, natural selection from predators is expected to outweigh sexual selection against signal synchrony. Considering this tradeoff, it is possible that eavesdropping predators impose high selective pressure that results in signal display strategies that are suboptimal for female attraction (extreme predator selection hypothesis). Pug-nosed tree frogs, for example, are one of the few anuran species that breed in the dry season (Heyer 1976), making them an important source of food for frog-eating predators during this time. Thus, pug-nosed tree frogs could experience higher selective pressure from eavesdropping predators than other frogs in the community. It is also possible, however, that relaxed selection from females allows production of signal display strategies that minimize exploitation by eavesdropping predators (relaxed sexual selection hypothesis). These hypotheses are not mutually exclusive. To our knowledge, this is the first study to integrate the effects of

target and multiple nontarget receivers to understand signaling strategies. We conduct both field and laboratory phonotaxis experiments to assess the preferences of female frogs and eavesdropping predators for synchronized versus alternating call timing strategies. We discuss our results in the context of the role of natural enemies and constraints imposed by mates on the evolution of ornament signals.

1.3 Methods

1.3.1 Risk of predation by frog-eating bats

Field experiments assessing eavesdropper attraction were conducted at eight locations in the forest around Gamboa, Republic of Panama (9°07.0'N, 79°41.9'W) during the breeding seasons of pug-nosed tree frogs (January to February 2015) and túngara frogs (June to August 2014). All locations were at least 1 km apart to minimize the chances that the same bats would be sampled at different locations as *T. cirrhosus* are known to have home range sizes < 1 km² (Jones et al. 2017). All locations were also within 0.5 km of a water source with breeding frogs, but also at a distance from where sounds produced by the water source or calling frogs was not audible.

At each of the eight field locations, three speaker stations were positioned 10 m apart from each other in a triangle formation to ensure equidistance between treatments. Speaker stations were placed at relative distance that resemble the spatial distribution of male frogs calling in a chorus but at distances and broadcasting patterns that minimized acoustic interference. At each station, two Pignose portable amplifier speakers (Model 7-100; Pignose-Gorilla, North Las Vegas, Nevada) were placed 1 m apart from each other, facing upwards. Each pair of speakers broadcast a pair of pre-recorded natural male calls with one of three degrees of temporal overlap: (i) near-perfect synchrony (5 ms of latency), (ii) natural average synchrony (79 ms of latency), or (iii) out of synchrony (alternating calls). These three treatments (5 ms, 79 ms, alternating) were thus presented simultaneously within a location (see Figure 1.1 for diagrams experimental set-ups). One, out of the eight locations, was tested in this way per night. The eight locations were rotated through three times for a total sampling period of 24 night, such that all three of the call timing treatments were tested at each of the three stations within each location.

For all treatments, frog calls were broadcast at a rate of one call every 2 s, at an amplitude of 82 dB SPL re. 20 μ P at 1 m from the speaker measured at ground level using a digital sound pressure level meter (Radio Shack catalogue number 33-2055; C-weighting, fast RMS response). We presented the calls of both frog species at a single standardized call rate and amplitude since our question was solely about differences in eavesdropper attraction resulting from the relative timing between calls. Additionally, only playbacks of simple, single note, calls for both pug-nosed tree frogs and túngara frogs were used to control for any confounding effect from signal complexity (Figure 1.2a,b). Calls for both species were randomly selected from a pre-recorded library of ten different individual males. For each treatment within a night, two calls were drawn from the library without replacement, and specific call combinations were not repeated for a receiver (Figure 1.3).

To compare the selective pressure imposed by frog-eating bats on different signal timing strategies, we video recorded bat attraction to the speakers in the field. At each of the three speaker stations, an infrared video camera (Bell and Howell model DNV16HDZ) was positioned 2 m from the two-speaker set and focused on the speaker broadcasting “following” calls. The behavior of the following male (calling in response to the “leader”) is responsible for call synchronization. As such, we were interested in the benefits that males may enjoy by producing following calls of different latencies. For the speaker set producing alternating calls, a focal speaker was chosen randomly from the two. A plastic model frog was placed on each speaker. For two hours immediately following sunset (Trillo et al. 2013), bat attraction to the speakers was video recorded. Following the protocol of other phonotaxis experiments with wild bats, videos were analyzed blind to treatment (Trillo et al. 2013; 2016). We quantified the attractiveness of a treatment by counting the number of bat “attacks” in a video, consisting of downwards flight towards the speaker in attempted prey capture. While we could not identify individual bats in the video, if certain treatments were more attractive to bats than others, the relative difference between treatments in the number of attacks should be maintained regardless of multiple visitations (Trillo et al. 2013; 2016). Additionally, as our collection methods between the pug-nosed tree frog and túngara frog breeding seasons were identical, we also compared the number of bat attacks between the dry and rainy season to determine differences in selective pressure from bats between the two frog species. Finally, even though other mammalian

predators were occasionally recorded in the area around the speakers (e.g. opossums and ocelots), bat attacks were the only recurrent “predation events” recorded.

1.3.2 Risk of attack by frog-biting midges

Immediately following each two-hour recording session quantifying predatory bat attraction, we assessed the attraction of frog-biting midges using the same field set-up and speaker station arrangement. We placed an acoustic trap (McKeever and Hartberg 1980) over the focal speaker at each station for a period of 45 min. These acoustic traps use a small fan that collects any small insects attracted to a speaker broadcasting calls. Following our bat experiment, all three signal timing treatments were presented within a location simultaneously and each of the eight locations was tested three times for a total sampling period of 24 nights. After the insects were collected, they were euthanized in the freezer overnight. Frog-biting midges were counted, identified to genus, and then preserved in 75% ethanol. We used the difference in the number of frog-biting midges attracted to the focal speaker between the call timing treatments as an indicator of acoustic preference. We also counted the number of individual mosquitoes (Culicidae) collected by the sound traps to identify possible additional eavesdroppers. Mosquitoes of the genus *Uranotaenia*, for example, are also known to use frog calls to find their hosts (Borkent and Belton 2006). As with the bat attraction experiment, we also compared the number of midges collected between the pug-nosed tree frog and túngara frog breeding seasons to determine differences in selective pressure from midges between the two frog species.

1.3.3 Attractiveness to female frogs

We collected frog pairs in amplexus from naturally occurring choruses during the breeding season for each species ($n = 23$ pug-nosed tree frogs in the dry season, January to March 2017, and $n = 40$ túngara frogs in the rainy season, October 2017). Female preference for conspecific calls broadcast at natural average synchrony (79 ms) or calls broadcast in alternation was tested in a $2\text{ m} \times 3\text{ m}$ semi-anechoic chamber. Calls were broadcast from two speaker stations, with each station containing two speakers. The speaker stations were spaced 3 m apart, with speakers spaced 1 m apart within the stations. The station playing each acoustic treatment, synchrony or alternation, was randomly selected for each female. Females were gently separated from the

male and positioned in the center of the chamber at 1.5 m from each of the two speaker stations under an acoustically transparent plastic cup. To ensure that female movement did not consist of escape behavior, females were given 1 min to adjust to the chamber before being remotely released. Mirroring the eavesdropper experiments, calls for both species were broadcast at 82 dB SPL re. 20 μ P at a rate of one call every 2 s. Following standard decision rules used in phonotaxis experiments with anurans (Gerhardt and Klump 1988; Ryan and Rand 1990), a choice was scored when the female approached a speaker within 10 cm without following the walls of the arena. Females were tested in each treatment once and both males and females were released together at the end of the night at their exact capture location.

To avoid retesting, we toe-clipped female túngara frogs prior to releasing them. Toe-clipping is a standard and efficient method to mark anurans (Donnelly et al. 1994). We avoided toe-clipping pug-nosed tree frogs, however, given that tree frogs heavily depend on their toepads for climbing. To identify female pug-nosed tree frogs, we built a photo library of all females captured over the course of the experiment. Both male and female pug-nosed tree frogs have pigmentation patterns on their backs that are individually distinctive (Duellman 1970), allowing for the successful implementation of this photo-based identification method.

1.3.4 Statistical analysis

All statistical analyses were conducted using Program R 3.5.2 (R Development Core Team 2013). To investigate the predation pressure imposed by bats and midges on different call timing strategies we compared eavesdropper attraction to calls produced in near-perfect synchrony (5 ms of latency), average synchrony (79 ms of latency), and out of synchrony (alternating calls). We used generalized linear mixed effect model (GLMM) functions in the *glmmTMB* package (Brooks et al. 2017) with a negative binomial error structure and a log link function (Trillo et al. 2016). Treatment was included as a fixed factor, site as a random factor, and date as a random factor nested within site. To determine differences among treatments, we performed a Tukey contrast test and calculated least squares means using the *emmeans* R package (Searle et al. 1980). Effect sizes, Cohn's *d*, were calculated using the *lsr* R package (Navarro 2015). We performed this analysis on the number of attacks for bats and number of midges or mosquitoes collected. These analyses were used to examine the effect of signal overlap in the calls of pug-

nosed tree frogs and túngara frogs independently. As comparisons of eavesdropper attraction were performed within each night, those nights without any bat attacks or no midges captured are uninformative were thus removed from the respective analyses (Trillo et al. 2016). The number of bat attacks and the number of midges collected were compared between the pug-nosed tree frog and túngara frog breeding seasons using a permutation test in the *coin* R package (Hothorn et al. 2008), with bat attacks or number of midges grouped within the eight sampling locations. We analyzed female preference for either calls broadcast at natural average synchrony or alternating calls using a two-tailed binomial test.

1.4 Results

1.4.1 Risk of predation by frog-eating bats

Field playback experiments using both pug-nosed tree frog and túngara frog calls were successful at attracting bats. Across all three treatments, a total of 343 general bat sightings were recorded during the playbacks of pug-nosed tree frog calls. The majority of those sightings involved bats passing by the area recorded by the camera and 54 were attacks (an average attack rate of 2.25 ± 0.65 per night). For túngara frog calls, a total of 398 general bat sightings were recorded, of which 65 were attacks (an average of 2.71 ± 1.14 per night). Between these two frog species, during their respective breeding seasons, the number of attacks by bats did not significantly differ ($Z = -0.52$, $p = 0.602$). The rate of bat observations reported here is similar to what has been observed in other studies examining bat phonotaxis in the wild (Trillo et al. 2016).

For pug-nosed tree frog calls, bat attacks were observed on 15 of the 24 nights. When cueing on pug-nosed tree frog calls, bats were more likely to attack speakers broadcasting alternating calls, as calls broadcast at near-perfect synchrony (5 ms latency) received on average about a quarter of the attacks of calls broadcast in alternation (0.53 ± 0.27 vs. 1.93 ± 0.49 attacks per night, $t(39) = -2.85$, $p = 0.019$, $d = 0.73$, Figure 1.4a). Other comparisons between call timings were not significantly different ($p > 0.05$). For túngara frog calls, bat attacks were observed on 11 of the 24 nights, and bats were equally likely to attack speakers of any of the three treatments ($p > 0.05$ for all combinations, Figure 1.4b).

1.4.2 Risk of attack by frog-biting midges

A total of 1,253 and 9,052 frog-biting midges were attracted to pug-nosed tree frog and túngara frog calls, respectively. Thus, between these two species, significantly more midges were attracted to túngara frog calls than pug-nosed tree frog calls during each species' breeding season ($Z = -0.52$, $p < 0.001$, $d = 0.94$). For pug-nosed tree frog calls, midges were captured on 23 out of the 24 nights, but there were no significant differences in the number of midges captured between any of the treatments ($p > 0.05$ for all combinations, Figure 1.4c). For túngara frog calls, midges were captured on all 24 nights, and calls broadcast in alternation attracted significantly more midges per night compared to calls broadcast at near-perfect synchrony (149.08 ± 35.70 vs. 104.25 ± 33.12 midges, $t(66) = -2.81$, $p = 0.018$, $d = 0.23$, Figure 1.4d). No other comparisons between traps broadcasting calls with different timing were significant ($p > 0.05$). A full list of the Tukey comparisons for the eavesdropper experiments is included in the electronic supplementary material (Table 1.1).

Additionally, an average of 4.29 and 16.67 mosquitoes (Culicidae) were collected per night for pug-nosed tree frog and túngara frog calls, respectively. A small proportion ($< 2\%$) of the mosquitoes in the acoustic traps were *Uranotaenia lowii*, a species known to acoustically orient to frog calls (Borkent and Belton 2006). There was, however, no significant difference in the number of mosquitoes attracted to different treatments ($p > 0.05$ for all combinations) for the calls of either frog species.

1.4.3 Attractiveness to female frogs

Female pug-nosed tree frogs were indifferent to speakers broadcasting alternating calls versus synchronized ones, with 15 of the 26 tested females choosing alternating calls (two-tailed binomial test: $p = 0.557$, Figure 1.5a). In contrast, female túngara frogs displayed a strong preference, with a higher proportion of females choosing alternating calls (33 of 40, two-tailed binomial test: $p < 0.001$, Figure 1.5b). Using the effect size of the female túngara choice experiment for comparison, the pug-nosed tree frog experiment had adequate statistical power ($1 - \beta = 0.93$, $\alpha = 0.05$, effect size = 0.325; using G*Power 3.1, Faul et al. 2009).

1.5 Discussion

Synchronization of mating signals is an evolutionary puzzle, given the assumed high cost of reduced female attraction when signals overlap. Synchronization may be beneficial, however, if overlapping signals reduce attraction of nontarget receivers. We found that synchronized pug-nosed tree frog calls attract fewer frog-eating bats than unsynchronized signals. Similarly, synchronized túngara frog calls attract fewer frog-biting midges than calls produced in alternation. These findings support the eavesdropper avoidance function of synchronized signals, first proposed by (Tuttle and Ryan 1982). Furthermore, by testing the calls of two frog species, one that naturally synchronizes its calls (pug-nosed tree frogs) and one that calls antiphonally (túngara frogs), we find that the eavesdropper avoidance benefit of synchronization is not limited only to synchronizing species.

1.5.1 Signal synchronization in the context of multiple eavesdroppers

While we found decreased eavesdropper attack rates in response to synchronous calling, bats and midges responded differently to call timing for each frog species. Synchronized pug-nosed tree frog calls attracted fewer bats but did not affect midge attraction, while synchronized túngara frog calls attracted fewer midges but did not affect bat attraction. We propose that the species-specific differences in eavesdropper attraction are likely due to differences in spectral and temporal properties of each frog species' calls (Tuttle and Ryan 1982; Ryan 1985; Figure 1.2). In addition, we expect such diverse eavesdroppers to greatly differ in the way acoustic signals are received and processed. How each receiver perceives such signals, however, is still a mystery (Page et al. 2014). Further studies that investigate the physiology of the auditory systems of these eavesdroppers are necessary to confirm the relationship between call timing and other call properties in reducing eavesdropper attraction.

In both eavesdropper experiments, only calls presented in near-perfect synchrony significantly reduced the attraction of bats or midges, with no difference between calls broadcast at average synchrony and antiphonally. It is likely that eavesdropper preference decreases as a function of the degree of synchrony. To better understand the ecological relevance of the delay between signals of neighboring males, further studies assessing eavesdropper preference along a finer

gradient of latencies is needed. Given that males of many frog species, including those in this study (da Silva Nunes 1988; Page and Ryan 2005), can alter their calling behavior in response to perceived increased predation risk, it is possible that frogs plastically increase the degree of signal overlap to further decrease signal conspicuousness. Studies that examine plasticity of fine timing responses of males signaling in choruses and how they are modulated by perceived predation risk would provide valuable insights to further understand the evolution of signal synchronization.

1.5.2 Implications for the origin and maintenance of signal synchronization

While our results suggest that call synchronization can reduce eavesdropper attraction, we found no evidence of greater predation risk from bats on pug-nosed tree frogs compared to túngara frogs. In both species, calling males are attacked at similar rates by frog-eating bats. As mentioned above, however, male pug-nosed tree frogs calling in synchrony benefit from a reduction in bat attacks, a benefit absent for túngara frogs. In contrast, frog-biting midges attacked túngara frogs in much greater numbers than pug-nosed tree frogs. The immediate lethal effects of bat attacks compared to the slower, additive effects of midge attacks suggests that pug-nosed tree frogs enjoy a larger benefit than túngara frogs when their calls are produced in synchrony. It is thus possible that a larger benefit of obscuring the calls by synchronizing them with neighboring males could have favored call synchronization in pug-nosed tree frogs. Overall, however, given the rates of bat and midge attraction across seasons, our results suggest that male pug-nosed tree frogs synchronize even though the general selective pressure imposed by eavesdroppers is similar to the levels experienced by other frog species in the community. That is, despite being one of the few anuran species calling in the dry season (Heyer 1976), pug-nose tree frogs are not attacked more by bats, and are even attacked less by midges, than túngara frogs. Therefore, higher predation pressure is unlikely to have been a major driver of call synchronization, and the extreme predator selection hypothesis is not supported. Instead, the key to signal synchronization may be in the selective preferences of a different receiver in the communication network, the target receiver of mating calls, conspecific females.

The responses of females to calls with different timing relative to calls of their neighbors revealed species-specific differences in their preferences. Our study confirmed that female

túngara frogs, the non-synchronous species, prefer calls broadcast antiphonally over calls that are synchronized (but see Schwartz and Rand 1991). This preference for calls out of synchrony is assumed to be a general strategy across anuran species given that females select signals in aggregations where overlapping signals impose a cognitive challenge for localizing and discriminating individual signals (Schwartz 1987; Bee and Micheyl 2008). It is this preference for unmasked calls that is assumed to drive that use of non-synchronous, alternating calls as a general strategy in frogs (Wollerman 1999; Wollerman and Wiley 2002a; Wollerman and Wiley 2002b), including the *Smilisca* clade (Figure 1.6). Female pug-nosed tree frogs, however, have no such preference and deviate from the general strategy of preferring calls produced out of synchrony. Such lack of preference suggests a reduction in the strength of preference for non-overlapping calls in pug-nosed tree frogs, resulting in a shift in the tradeoff of selective pressures on synchronization. For male pug-nosed tree frogs, the selective pressure against producing following calls is lower than for túngara frogs. These findings support the idea that relaxed selection by females has provided the opportunity for signaling males to synchronize their calls and thus reduce attacks by eavesdroppers (relaxed sexual selection hypothesis). While traditional models of female preference and the evolution of mating signals have emphasized positive selection (e.g. Haldane 1932; Prout 1964; Fong et al. 1995), there is recent increased attention to the role of relaxed selection in the maintenance and evolution of traits in general (Lahti et al. 2009). Relaxation of selection ultimately shifts the relationship between costs and benefits, potentially shaping trait tradeoffs and resulting in trait evolution. Male anuran signal timing is influenced by a tradeoff between eavesdropper and female attraction (Page et al. 2014). Yet, despite experiencing similar levels of predation pressure, pug-nosed tree frogs are one of the few anurans to produce near-perfectly synchronized calls in this community. Overall, our results suggest that male pug-nosed tree frogs are released from a cost imposed by reduced female attraction. We, therefore, propose that while avoidance of eavesdroppers is the function of synchronized signaling, relaxation of female preference for unsynchronized signals has allowed for the evolution and maintenance of a synchronized signal timing strategy in pug-nosed tree frogs. It is still unclear, however, if female pug-nose tree frogs struggle with challenges associated with localizing and discriminating between mates as females from many other anuran species do (Wollerman 1999; Wollerman and Wiley 2002a; Wollerman and Wiley 2002b).

Further studies that examine the ability of female pug-nosed tree frogs to localize and discriminate between preferred males calling in synchrony would provide valuable insights.

1.5.3 Other functions of signal synchronization in anurans and other taxa

Other drivers, in addition to avoidance of eavesdroppers, may selected for call synchronization. For instance, synchrony may arise through male-male competition, where a male may try to mask a neighbor's call with his own call reducing his neighbor's attractiveness (Wells 1977; Greenfield 1994). Given that by masking a neighbor's call the synchronizing male also masks his own call reducing his own attractiveness, this function seems unlikely (Wells 1977). If features at the end of the call increase female attraction, however, a synchronizing male may be able to preserve the attractiveness of his call while still masking his neighbor's call. In hourglass tree frogs (*Dendropsophus ebraccatus*) and African running frogs (*Kassina fusca*), for example, males produce complex multi-note calls and females prefer calls with unobstructed ends. In both species, males overlap calls with neighboring males resulting in following males masking the tail end of the leader's call, while the end of the follower's call remains unobstructed (Wells and Swartz 1984; Grafe 1999). In comparison, the calls of pug-nosed tree frogs lack distinct features towards the end that a following male would benefit from obstructing. Therefore, due to the simplicity of the calls of pug-nosed tree frog, synchrony through male-male competition seems unlikely.

Synchronized calling may also benefit males by increasing the peak amplitude of their combined calls through constructive interference. Groups of synchronized males may create a "beacon", increasing the active space of their signals compared to a group of unsynchronized males (Buck and Buck 1978). First proposed in fireflies, this "beacon effect" hypothesis has been proposed to explain synchronization of acoustic signals in insect (Shelly and Greenfield 1991; Greenfield 1994) and anuran (Wells 1977) choruses but has not been directly tested in the latter. The beacon effect hypothesis, similar to the eavesdropper hypothesis, comes with a potential cost to female attraction. As with almost any trait related to chorusing, the per capita increase in female attraction to the chorus must outweigh the cost of reduced female attraction to an individual male within the chorus (Ryan et al. 1981). In the context of the beacon effect, it is also unclear how this calling strategy is resistant to cheaters, since a non-synchronous male producing

unobstructed calls would enjoy the benefits of increased numbers of females attending the chorus but would be more attractive to females once they reach the chorus. There are, however, particular habitats that may limit the benefits gained by cheating. In habitats with high levels of background noise, such as waterfalls or streams, the calls of a single male may already be acoustically masked. Overcoming high levels of background noise could thus lead to call synchronization as an evolutionary stable strategy that takes advantage of the beacon effect (Marshall and Gerhardt unpublished data reviewed in Gerhardt and Huber 2002). Consistent with this idea, male pug-nosed tree frogs form choruses around waterfalls and torrents of streams (Nunes 1988) in which the dominant frequency of their call overlaps with the background noise generated by the running water (Tuttle and Ryan, 1982; da Silva Nunes, 1988). This acoustic masking suggests males of this species could benefit from the beacon effect to attract females. An increased active space of the chorus, however, will also result in increased attraction of eavesdropping predators. Further studies are needed that examine the potential role of the beacon effect on call synchronization, while also considering the effect of eavesdroppers.

Finally, while synchronization is rare in frogs and toads, having only been identified in a handful of anuran species in addition to pug-nosed tree frogs (*Kassina senegalensis*: Wickler and Seibt 1974; *D. ebraccatus*: Wells and Schwartz 1984; *Cochranella granulose*: Ibáñez 1993; *K. fusca*: Grafe 1999; *Kassina kuvangensis*: Grafe 2003; *Hyla arenicolor*: Marshall and Gerhardt unpublished data reviewed in Gerhardt and Huber 2002; *Assa darlingtoni*: Clulow et al. 2017; *Diasporus diastema*: Capshaw et al. 2018), this signal timing strategy has been observed across diverse taxonomic groups. Synchronization is a common strategy for many insects, such as crickets and katydids, that form large nocturnal choruses that share many similarities to anuran choruses (Walker 1969; Shaw et al. 1990; Sismondo 1990; Greenfield and Roizen 1993; Nityanand and Balakrishnan 2007; Greenfield and Schul 2008; Schul et al. 2014). In such large multispecies choruses, synchrony may benefit males by maintaining a species-specific rhythm, allowing females to more easily identify conspecifics (Greenfield 1994; Moiseff 2010).

Signal synchronization in groups other than anurans and insects is used in different contexts. Male and female birds, for example, may synchronize mating signals in the form of duets (Hall 2009). Unlike insect and anuran choruses, however, synchronization in bird songs function primarily in mate and territory defense rather than as a mate attraction strategy. The

synchronized howling of wolves (*Canis lupus*) and coyotes (*Canis latrans*) also plays a role in territorial maintenance (Harrington and Mech 1979; McCarley 1975) through the Beau Geste effect (Harrington 1989), similar to the beacon effect, in which synchronizing masks a pack's size or make it appear larger to distant receivers. Synchronization is also observed in other signal modalities, such as in the luminescent displays produced by some species of fireflies (Buck and Buck 1978) and marine ostracods (Morin 1986). Whether these visual displays help mask individuals from eavesdropping predators, however, has yet to be tested.

1.6 Conclusion

Predators have often been invoked as a driving force that curtails exaggeration of mating signals (Andersson 1994), but evolutionary biologists have devoted less attention to consider how relaxed selection by females can allow signalers to escape eavesdropper exploitation of their communication system. Additionally, to date most studies on relaxed selection have focused on non-sexual selective pressures, such as predator release or abiotic changes in the environment (Lahti et al. 2009). To our knowledge, this study provides the first example of how relaxed female choice may result in trait evolution in nature. Our results bring to light the complex nature of tradeoffs and the role of relaxed selection at promoting the evolution of unique signaling strategies.

Table 1.1. Tukey comparisons for bat attacks on pug-nosed tree frog (a) and túngara frog (b) calls, and midges attracted to pug-nosed tree frog (c) and túngara frog (d) calls. Table corresponds to Figure 1.4. The values for least squares means estimate, standard error, *t*-ratio, and *p*-value were calculated using the *emmeans* R package. Cohn's *d* effect size was calculated using the *lsr* R package.

	Least squares means	Standard error	<i>t</i> -ratio	<i>p</i> -value	Cohn's <i>d</i> effect size
(a) Bat attacks on pug-nosed tree frog calls (df = 39)					
5ms - 79ms	-0.746	0.486	-1.536	0.286	0.307
5ms - alt	-1.305	0.459	-2.846	0.019 *	0.729
79ms - alt	-0.560	0.378	-1.482	0.310	0.439
(b) Bat attacks on túngara frog calls (df = 27)					
5ms - 79ms	-0.144	0.402	-0.358	0.932	< 0.001
5ms - alt	0.157	0.412	0.380	0.924	0.223
79ms - alt	0.301	0.414	0.728	0.749	0.128
(c) Midges attracted to pug-nosed tree frog calls (df = 63)					
5ms - 79ms	0.047	0.260	0.182	0.982	0.121
5ms - alt	-0.412	0.256	-1.609	0.242	0.249
79ms - alt	-0.459	0.250	-1.835	0.402	0.166
(d) Midges attracted to túngara frog calls (df = 66)					
5ms - 79ms	-0.232	0.199	-1.166	0.478	0.184
5ms - alt	-0.559	0.199	-2.814	0.018 *	0.228
79ms - alt	-0.327	0.195	-1.682	0.220	0.164

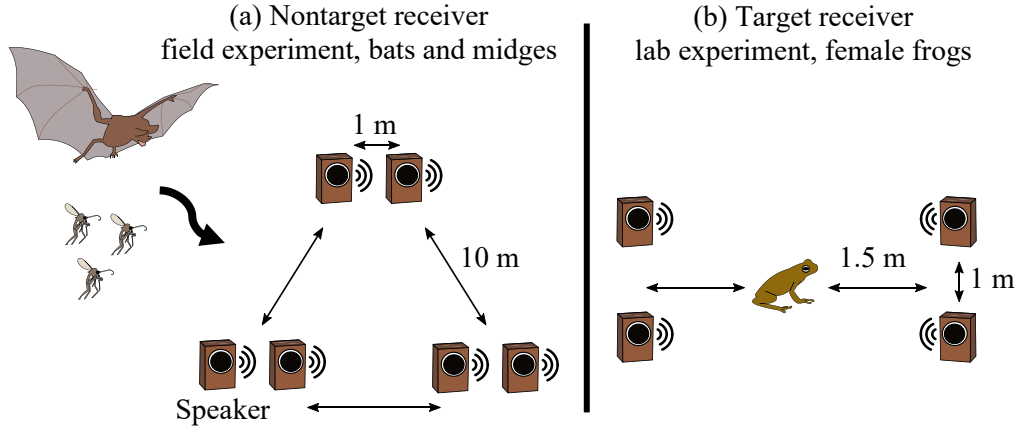


Figure 1.1. Experimental set-ups for the receiver phonotaxis experiments. For the eavesdropper, nontarget receiver experiments (a), three stations of two speakers were positioned 10 m apart. Each of the three speaker stations simultaneously broadcast one of the three treatments: (i) calls at near-perfect synchrony (5 ms of latency), (ii) calls at natural average synchrony (79 ms of latency), or (iii) calls out of synchrony (alternating calls). Eavesdropping midge and bat attraction to the focal speaker (the speaker broadcasting “following” calls) was compared across treatments. For the target receiver experiment (b), two speaker stations were positioned 3 m apart in a 2 m×3 m semi-anechoic chamber. Each station broadcast either (i) synchronized calls (79 ms of latency) or (ii) alternating calls. The near-perfect synchrony treatment (5 ms latency) was not included in the target receiver experiment because the semi-anechoic sound chamber was restricted to 4 speakers (2 speaker station choice tests). Of the two synchrony treatments, calls broadcast with the average latency (79 ms) was chosen.

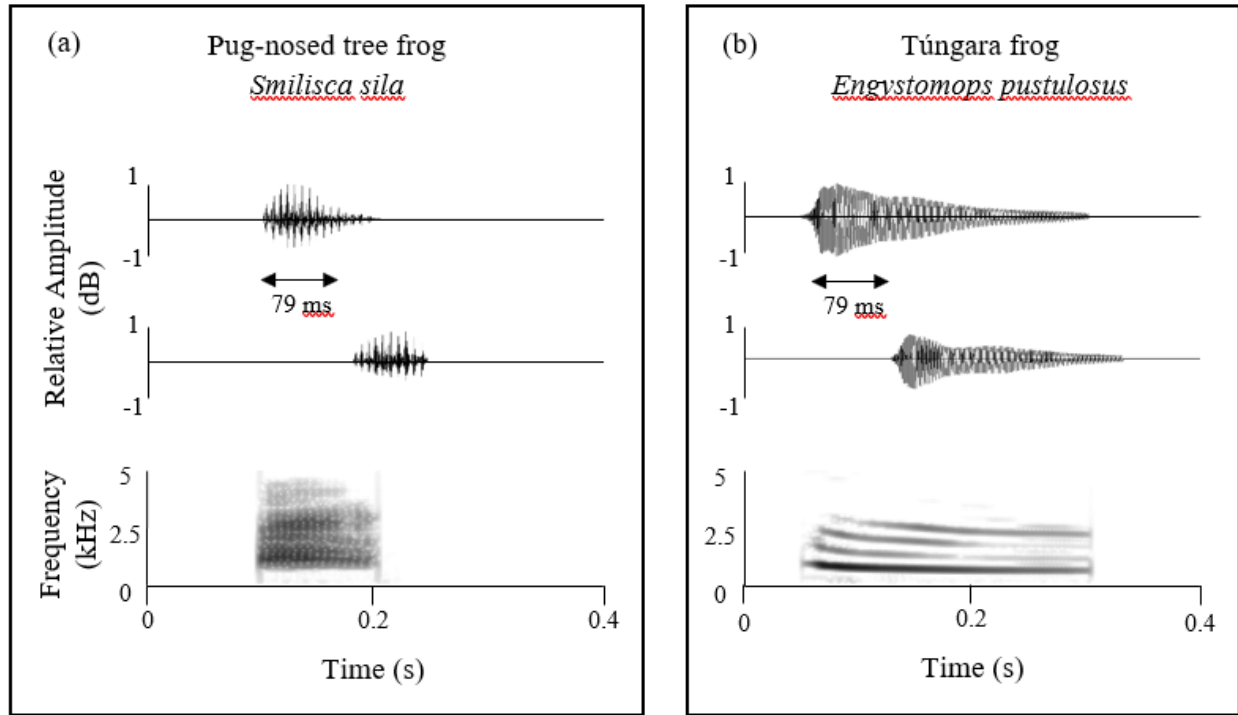
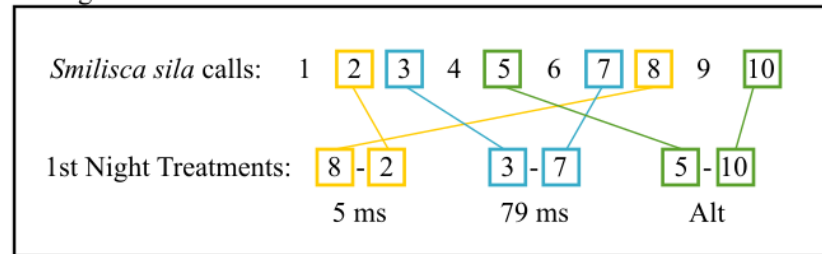
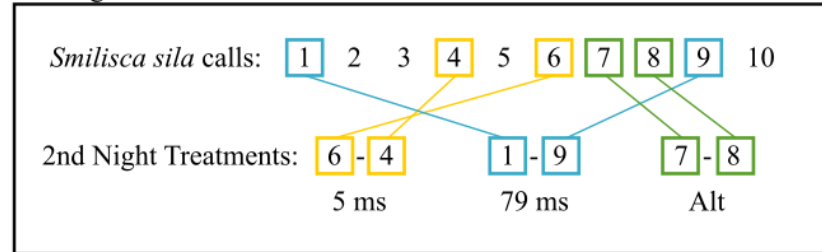


Figure 1.2. Oscillogram (top) and spectrogram (bottom) of the mating calls of the pug-nosed tree frog (a) and túngara frog (b). The oscillogram shows two calls synchronized with a latency of 79 ms, the average natural synchrony of pug-nosed tree frogs. The spectrogram shows only the first call. Although both pug-nosed tree frogs and túngara frogs can produce a more complex, multi-note call, only playbacks of simple calls (as shown) were used in this study.

1st Night



2nd Night



3rd Night

24th Night

Figure 1.3. Method for creating stimuli, treatments (5 ms, 79 ms, alternating calls) for each trial. Pre-recorded calls were randomly drawn from the library of 10 (either *Smilisca sila* or *Engystomops pustulosus*) so that the same call was not repeated within a single trial. Specific call combinations were not repeated for a receiver (bat, midge, or female frog). Note that as a result of leader-follower preferences [1], the order in which calls are presented in the 5 ms and 79 ms treatments change the way these stimuli are perceived (e.g. call #8 and call #2 broadcast at 5 ms overlap is different than call #2 and call #8 broadcast at 5 ms overlap). Thus, from the library of 10 calls, there were 90 potential stimuli each for the 5 ms and 79 ms treatments, and 45 combinations for the alternative treatment.

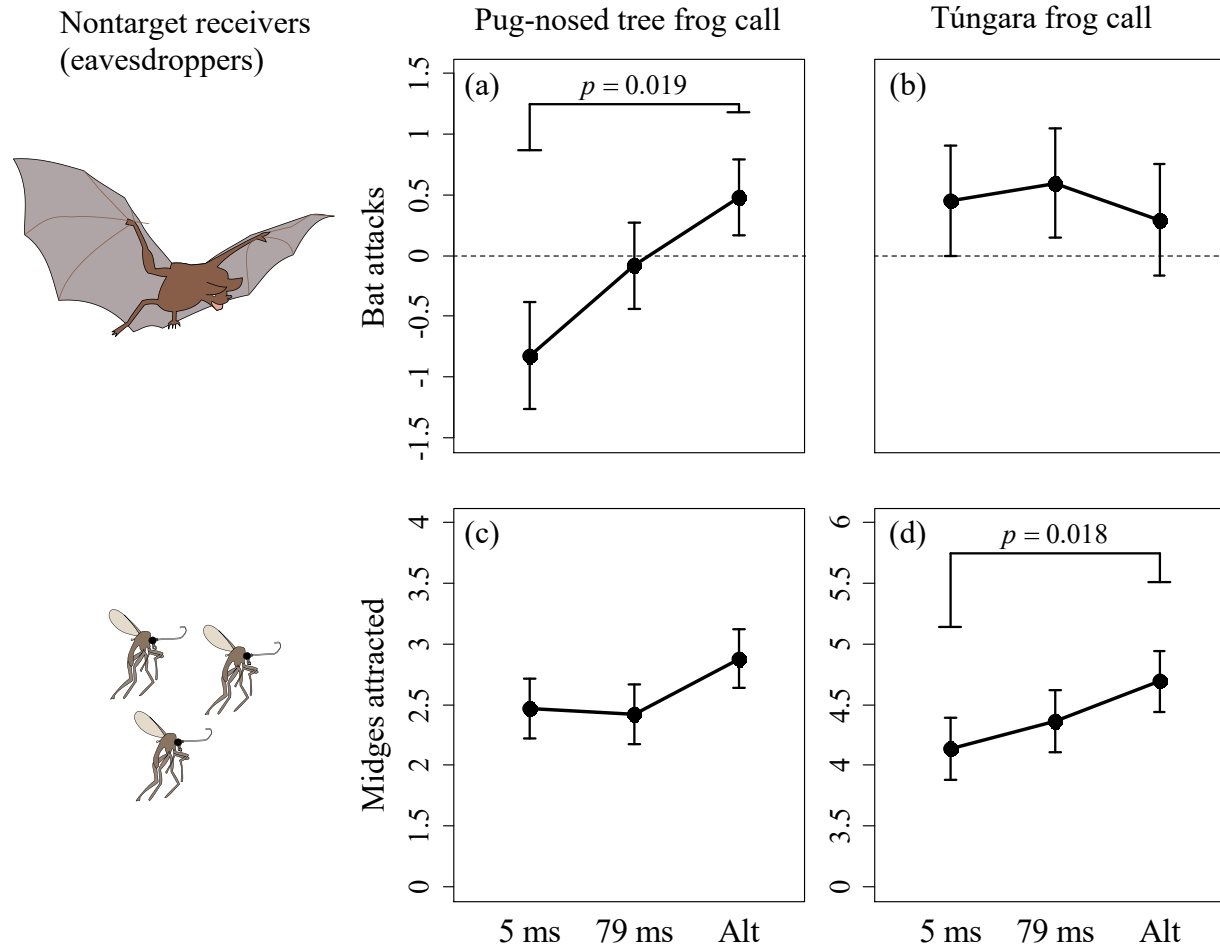


Figure 1.4. Eavesdropper preferences for synchronized and unsynchronized calls, measured for both pug-nosed tree frog (left) and túngara frog (right) calls during each species' respective breeding season. Number of bat attacks per night (a,b) and number of midges captured per night (c,d) for calls were compared for different call timings: near-perfect synchrony (5 ms of latency), average pug-nosed tree frog call synchrony (79 ms of latency) and alternating calls (Alt). Values are the least squared means and bars show standard error. Note that for midge attraction, the y-axis range for pug-nosed tree frogs (c) is different than túngara frogs (d).

target receivers
(females)

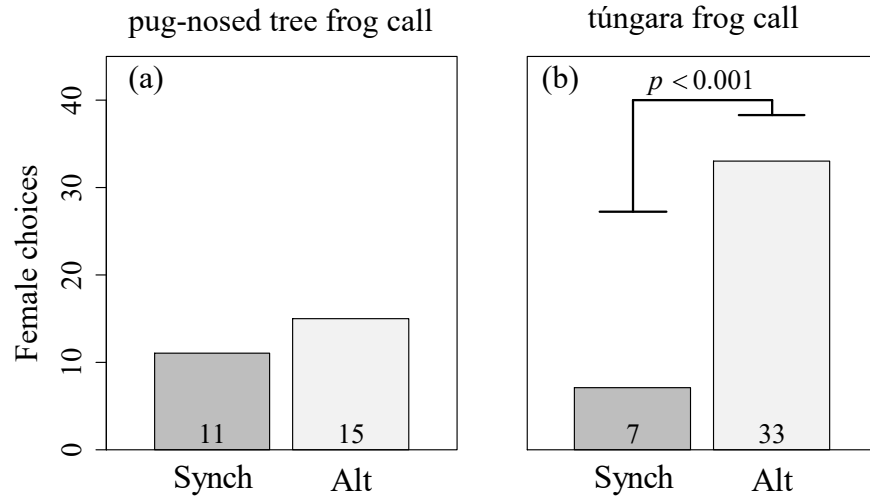
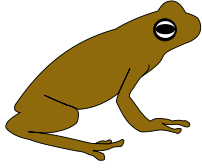


Figure 1.5. Female preference for synchronized and unsynchronized calls, measured for both pug-nosed tree frog (a) and túngara frog (b) calls during each species' respective breeding season. Female choice was compared between calls broadcast in synchrony with 79 ms of latency between calls (Synch), or broadcast in alternation (Alt). Each choice represents a single female.

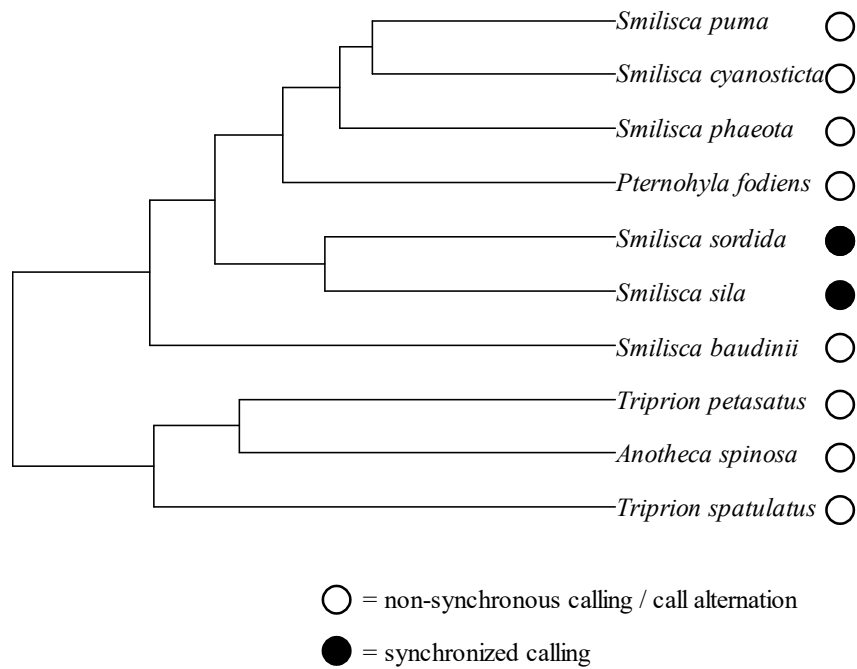


Figure 1.6. Phylogeny of *Smilisca* clade (Smith et al. 2007). Circles following each species name show call timing strategy, assigned based on literature (Savage 2002) and from call recordings. While *S. sordida*, the sister species of *S. sila*, is described as having alternating calls, male *S. sordida* often call in a rapid sequential “domino” pattern (p. 357 in Savage 2002) that can result in signal overlap. Open circles indicate non-synchronous calling, black circles indicate the use of synchronized calls, and gray circles indicate an intermediate calling strategy: alternating calls with some overlap.

1.7 References

- Andersson, M. B. (1994). *Sexual selection*. Princeton University Press, Princeton.
- Bee, M. A., and Micheyl, C. (2008). The cocktail party problem: what is it? How can it be solved? And why should animal behaviorists study it?. *Journal of Comparative Psychology* 122: 235-251.
- Bernal, X. E., and Pinto, C. M. (2016). Sexual differences in prevalence of a new species of trypanosome infecting túngara frogs. *International Journal for Parasitology: Parasites and Wildlife* 5: 40-47.
- Bernal, X. E., Rand, A. S., and Ryan, M. J. (2006). Acoustic preferences and localization performance of blood-sucking flies (*Corethrella* Coquillett) to túngara frog calls. *Behavioral Ecology* 17: 709-715.
- Borkent, A., and Belton, P. (2006). Attraction of female *Uranotaenia lowii* (Diptera: Culicidae) to frog calls in Costa Rica. *The Canadian Entomologist* 138: 91-94.
- Bosch, J., and Márquez, R. (2002). Female preference function related to precedence effect in an amphibian anuran (*Alytes cisternasii*): tests with non-overlapping calls. *Behavioral Ecology* 13: 149-153.
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., and Bolker, B. M. (2017). *glmmTMB* balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* 9: 378-400.
- Buck, J., and Buck, E. (1978). Toward a functional interpretation of synchronous flashing by fireflies. *The American Naturalist* 112: 471-492.
- Camp, J. V. (2006). *Host attraction and host selection in the family Corethrellidae (Wood and Borkent)(Diptera)*. Unpublished master's thesis. Georgia Southern University, Statesboro.
- Capshaw, G., Foss-Grant, A. P., Hartmann, K., Sehuanes, J. F., and Moss, C. F. (2020). Timing of the advertisement call of the common tink frog (*Diasporus diastema*) shifts with the acoustic behaviour of local conspecifics. *Bioacoustics* 29: 79-96.
- Carlson, A. D., and Copeland, J. (1985). Flash communication in fireflies. *The Quarterly review of biology* 60: 415-436.
- Clulow, S., Mahony, M., Elliott, L., Humfeld, S., and Gerhardt, H. C. (2017). Near-synchronous calling in the hip-pocket frog *Assa darlingtoni*. *Bioacoustics* 26:249-258.
- da Silva Nunes, V. (1988). Vocalizations of treefrogs (*Smilisca sila*) in response to bat predation. *Herpetologica* 44: 8-10.
- Donnelly, M. A., Guyer, C., Juterbock, E. J., and Alford, R. A. (1994). Techniques for marking amphibians. In *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. (eds. W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster). pp. 275-284. Smithsonian Institution Press, District of Columbia.
- Duellman, W. E. (1970). *Hylid frogs of Middle America*. University of Kansas Press, Lawrence.
- Farabaugh, S. M. (1982). The ecological and social significance of duetting. In *Acoustic communication in birds, Vol. 2 Song learning and its consequences*, (eds. D. E. Kroodsma and E. H. Miller) pp. 85-124. Academic Press, New York.
- Faul, F., E. Erdfelder, A. Buchner, and Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods* 41: 1149-1160.
- Fong, D. W., Kane, T. C., and Culver, D. C. (1995). Vestigialization and loss of nonfunctional characters. *Annual Review of Ecology and Systematics* 26: 249-268.

- Gerhardt, H. C., and Huber, F. (2002). *Acoustic communication in insects and anurans: common problems and diverse solutions*. University of Chicago Press, Chicago.
- Gerhardt, H.C., and Klump G.M. (1988). Masking of acoustic signals by the chorus background noise in the green tree frog: A limitation on mate choice. *Animal Behaviour* 36: 1247-1249.
- Grafe, T. U. (1996). The function of call alternation in the African reed frog (*Hyperolius marmoratus*): precise call timing prevents auditory masking. *Behavioral Ecology and Sociobiology* 38: 149-158.
- Grafe, T. U. (1999). A function of synchronous chorusing and a novel female preference shift in an anuran. *Proceedings of the Royal Society B* 266: 2331-2336.
- Grafe, T. U. (2003). Synchronized interdigitated calling in the Kuvangu running frog, *Kassina kuvangensis*. *Animal Behaviour* 66: 127-136.
- Greenfield, M. D. (1994). Synchronous and alternating choruses in insects and anurans: common mechanisms and diverse functions. *American Zoologist* 34: 605-615.
- Greenfield, M. D., and Roizen, I. (1993). Katydid synchronous chorusing is an evolutionarily stable outcome of female choice. *Nature* 364: 618-620.
- Greenfield, M. D., and Schul, J. (2008). Mechanisms and evolution of synchronous chorusing: emergent properties and adaptive functions in *Neoconocephalus* katydids (Orthoptera: Tettigoniidae). *Journal of Comparative Psychology* 122: 289-297.
- Greenfield, M. D., Tourtellot, M. K., and Snedden, W. A. (1997). Precedence effects and the evolution of chorusing. *Proceedings of the Royal Society B* 264: 1355-1361.
- Haldane, J. B. S. (1932) *The causes of evolution*. Longmans, Green. (Revised ed. 1990). Princeton University Press. Princeton.
- Hall, M. L. (2009). A review of vocal duetting in birds. *Advances in the Study of Behavior* 40: 67-121.
- Harrington, F. H. (1989). Chorus howling by wolves: acoustic structure, pack size and the Beau Geste effect. *Bioacoustics* 2: 117-136.
- Harrington, F. H., and Mech, L. D. (1979). Wolf howling and its role in territory maintenance. *Behaviour* 68: 207-249.
- Hebets, E. A., and Papaj, D. R. (2005). Complex signal function: developing a framework of testable hypotheses. *Behavioral Ecology and Sociobiology* 57: 197-214.
- Heyer, W.R. (1976). *Studies in Larval Amphibian Habitat Partitioning*. Smithsonian Institution Press, District of Columbia.
- Höbel, G. (2010). Interaction between signal timing and signal feature preferences: causes and implications for sexual selection. *Animal Behaviour* 79: 1257-1266.
- Hothorn T, Hornik K, van de Wiel MA, Zeileis A (2006). "A Lego system for conditional inference." *The American Statistician* 60: 257–263.
- Ibáñez, R. (1993). Female phonotaxis and call overlap in the neotropical glassfrog *Centrolenella granulosa*. *Copeia* 1993: 846-850.
- Johnson, R. N., Young, D. G., and Butler, J. F. (1993). Trypanosome transmission by *Corethrella wirthi* (Diptera: Chaoboridae) to the green treefrog, *Hyla cinerea* (Anura: Hylidae). *Journal of Medical Entomology* 30: 918-921.
- Jones, P. L., Hämsch, F., Page, R. A., Kalko, E. K., and O'mara, M. T. (2017). Foraging and roosting behaviour of the fringe-lipped bat, *Trachops cirrhosus*, on Barro Colorado Island, Panamá. *Acta Chiropterologica* 19: 337-346.

- Kirkpatrick, M., and Ryan, M. J. (1991). The evolution of mating preferences and the paradox of the lek. *Nature* 350: 33-38.
- Klump, G. M., and Gerhardt, H. C. (1992). Mechanisms and function of call-timing in male-male interactions in frogs. In *Playback and studies of animal communication*. (ed. P. K. McGregor), pp. 153–174. Plenum Press, New York.
- Lahti, D. C., Johnson, N. A., Ajie, B. C., Otto, S. P., Hendry, A. P., Blumstein, D. T., Cross, R. G., Donohue, K., and Foster, S. A. (2009). Relaxed selection in the wild. *Trends in ecology and Evolution* 24: 487-496.
- Legett, H. D., Baranov, V. A., and Bernal, X. E. (2018). Seasonal variation in abundance and diversity of eavesdropping frog-biting midges (Diptera, Corethrellidae) in a neotropical rainforest. *Ecological Entomology* 43: 226-233.
- Legett, H. D., Page, R. A., and Bernal, X. E. (2019). Data from: Synchronized mating signals in a communication network: the challenge of avoiding predators while attracting mates. Dryad Digital Repository. (doi:10.5061/dryad.rd03773)
- McCarley, H. (1975). Long-distance vocalizations of coyotes (*Canis latrans*). *Journal of Mammalogy* 56: 847-856.
- McGregor, P. K. (Ed.). (2005). *Animal communication networks*. Cambridge University Press, Cambridge.
- McGregor, P. K., and Peake, T. M. (2000). Communication networks: social environments for receiving and signalling behaviour. *Acta Ethologica* 2: 71-81.
- McKeever, S., and Hartberg, W. K. (1980). An effective method for trapping adult female *Corethrella* (Diptera: Chaoboridae). *Mosquito News* 20: 111-112.
- Moiseff, A., Copeland, J. (2010) Firefly Synchrony: A Behavioral Strategy to Minimize Visual Clutter. *Science* 329: 181–181.
- Morin, J. G. (1986). Firefleas of the sea: luminescent signaling in marine ostracode crustaceans. *Florida Entomologist* 69: 105-121.
- Navarro, D. (2015). *lsr: Companion to “Learning Statistics with R”*. University of Adelaide. Adelaide.
- Nityananda, V., and Balakrishnan, R. (2007). Synchrony during acoustic interactions in the bushcricket Mecopoda ‘Chirper’ (Tettigoniidae: Orthoptera) is generated by a combination of chirp-by-chirp resetting and change in intrinsic chirp rate. *Journal of Comparative Physiology A* 193: 51.
- Page, R. A. and Ryan, M. J. (2005). Flexibility in assessment of prey cues: frog-eating bats and frog calls. *Proceedings of the Royal Society B* 272: 841-847.
- Page, R. A., Ryan, M. J., and Bernal, X. E. (2014). Be loved, be preyed, be eaten. In *Animal behavior case studies: integration and application of animal behavior, Vol. 3*. (ed. K. Yasukawa). pp. 123-154. Praeger, New York.
- Peake, T.M. (2005). Eavesdropping in communication networks. In *Animal Communication Networks*. (ed. P.K. McGregor), pp 13–37. Cambridge Univ Press, Cambridge.
- Prout, T. (1964). Observations on structural reduction in evolution. *The American Naturalist* 98: 239-249.
- R Core Team (2013). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Reaney, L. T., Sims, R. A., Sims, S. W., Jennions, M. D., and Backwell, P. R. (2008). Experiments with robots explain synchronized courtship in fiddler crabs. *Current Biology* 18: R62-R63.

- Ryan, M. J. (1985). *The túngara frog: a study in sexual selection and communication*. University of Chicago Press, Chicago.
- Ryan, M. J. (1986). Synchronized calling in a treefrog (*Smilisca sila*). *Brain, Behavior and Evolution* 29: 196-206.
- Ryan, M. J., and Rand, A. S. (1990). The sensory basis of sexual selection for complex calls in the túngara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). *Evolution* 44: 305-314.
- Ryan, M. J., Tuttle, M. D., and Rand, A. S. (1982). Bat predation and sexual advertisement in a neotropical anuran. *The American Naturalist* 119: 136-139.
- Ryan, M. J., Tuttle, M. D., and Taft, L. K. (1981). The costs and benefits of frog chorusing behavior. *Behavioral Ecology and Sociobiology* 8: 273-278.
- Savage, J. M. (2002). *The amphibians and reptiles of Costa Rica: a herpetofauna between two continents, between two seas*. University of Chicago Press, Chicago.
- Schul, J., Bush, S. L., and Frederick, K. H. (2014). Evolution of call patterns and pattern recognition mechanisms in *Neoconocephalus* katydids. In *Insect hearing and acoustic communication*. (ed. B. Hedwig). pp. 167-183. Springer, Berlin.
- Schwartz, J. J. (1987). The function of call alternation in anuran amphibians: a test of three hypotheses. *Evolution* 41: 461-471.
- Schwartz, J. J., and Rand, A. S. (1991). The consequences for communication of call overlap in the Túngara frog, a neotropical anuran with a frequency-modulated call. *Ethology* 89: 73-83.
- Searle, S. R., Speed, F. M., and Milliken, G. A. (1980). Population marginal means in the linear model: an alternative to least squares means. *The American Statistician* 34: 216-221.
- Shaw, K. C., Galliard, P. L., and Smith, B. (1990). Acoustic behavior of *Amblycorypha parvipennis* (Orthoptera: Tettigoniidae). *Annals of the Entomological Society of America*, 83(3), 617-625.
- Shelly, T. E., and Greenfield, M. D. (1991). Dominions and desert clickers (Orthoptera: Acrididae): influences of resources and male signaling on female settlement patterns. *Behavioral Ecology and Sociobiology* 28: 133-140.
- Sismondo, E. (1990). Synchronous, alternating, and phase-locked stridulation by a tropical katydid. *Science* 249: 55-58.
- Taga, M. E., and Bassler, B. L. (2003). Chemical communication among bacteria. *Proceedings of the National Academy of Sciences* 100: 14549-14554.
- Trillo, P. A., Athanas, K. A., Goldhill, D. H., Hoke, K. L., and Funk, W. C. (2013). The influence of geographic heterogeneity in predation pressure on sexual signal divergence in an Amazonian frog species complex. *Journal of Evolutionary Biology* 26: 216-222.
- Trillo, P. A., Bernal, X. E., Caldwell, M. S., Halfwerk, W. H., Wessel, M. O., and Page, R. A. (2016). Collateral damage or a shadow of safety? The effects of signalling heterospecific neighbours on the risks of parasitism and predation. *Proceedings of the Royal Society B* 283: 20160343.
- Tuttle, M. D., and Ryan, M. J. (1982). The role of synchronized calling, ambient light, and ambient noise, in anti-bat-predator behavior of a treefrog. *Behavioral Ecology and Sociobiology* 11: 125-131.
- Walker, T. J. (1969). Acoustic synchrony: two mechanisms in the snowy tree cricket. *Science* 166: 891-894.
- Wells, K. D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour* 25: 666-693.

- Wells, K. D., and Schwartz, J. J. (1984). Vocal communication in a neotropical treefrog, *Hyla ebraccata*: advertisement calls. *Animal Behaviour* 32: 405-420.
- Whitney, C. L., and Krebs, J. R. (1975). Mate selection in Pacific tree frogs. *Nature* 255: 325-326.
- Wickler, W., and Seibt, U. (1974). Rufen und Antworten bei *Kassina senegalensis*, *Bufo regularis* und anderen Anuren. *Zeitschrift für Tierpsychologie* 34: 524-537.
- Wollerman, L. (1999). Acoustic interference limits call detection in a Neotropical frog *Hyla ebraccata*. *Animal Behaviour* 57: 529-536.
- Wollerman, L., and Wiley, H. R. (2002a). Possibilities for error during communication by neotropical frogs in a complex acoustic environment. *Behavioral Ecology and Sociobiology* 52: 465-473.
- Wollerman, L., and Wiley, R. H. (2002b). Background noise from a natural chorus alters female discrimination of male calls in a Neotropical frog. *Animal Behaviour* 63: 15-22.
- Zuk, M., and Kolluru, G. R. (1998). Exploitation of sexual signals by predators and parasitoids. *The Quarterly Review of Biology* 73: 415-438.

CHAPTER 2. PREY EXPLOITS THE AUDITORY ILLUSIONS OF EAVESDROPPING PREDATORS

H. D. Legett¹, C. T. Hemingway², X. E. Bernal^{1,3}

¹ *Purdue University, West Lafayette, IN, USA*

² *University of Texas, Austin, TX, USA*

³ *Smithsonian Tropical Research Institute, Balboa, Ancón, Panama, PA*

Citation

This chapter was originally published in the journal *The American Naturalist* and is reproduced here under the University of Chicago Press publication agreement.

Legett, H. D., Hemingway, C. T., and Bernal, X. E. (in press). Prey exploits the auditory illusions of eavesdropping predators. *The American Naturalist*.

2.1 Abstract

Mating signals have evolved to attract target receivers, even to the point of exploiting receivers through perceptual manipulation. Signals, however, can also expose signalers to non-target receivers, including predators and parasites, and thus have also evolved to decrease enemy attraction. Here we show that male treefrogs (*Smilisca sila*) reduce their attractiveness to eavesdropping enemies (bats and midges) by overlapping their calls at near-perfect synchrony with the calls of neighboring conspecifics. By producing calls that closely follow those of other males, synchronizing *S. sila* take advantage of an auditory illusion where enemies are more attracted to the leading call. Female *S. sila*, however, are less susceptible to this illusion. Thus, synchronization among signaling males can result in acoustic crypsis from predators without affecting female attraction. Given the widespread use of conspicuous mating signals and eavesdropping enemies, perceptual exploitation of eavesdroppers is likely a common driver of signal evolution.

2.2 Introduction

Mating signals can both evolve to advertise traits that benefit female reproductive success, as well as exploit pre-existing perceptual inaccuracies, without necessarily providing a direct benefit to the female (Ryan 1990; Christy 1995; Endler and Basolo 1998; Ryan and Cummings 2013). Females, however, are not the only receivers driving signal evolution. Mating signals also expose signalers to natural enemies, such as eavesdropping predators and parasites (Zuk and Kolluru 1998). While selective pressures imposed by female perceptual systems have received considerable attention (reviewed in Ryan and Cummings 2013), there has been little consideration of how exploiting perceptual misjudgments of eavesdroppers can also influence signal evolution.

Antagonistic selection from eavesdroppers can result in unexpected signal characteristics, such as reduced signal conspicuousness. A common example of this adaptation is the use of private communication channels between conspecifics in which the signal escapes the range of sensitivity of their predator, such as visual signaling in the ultraviolet range (Cummings et al. 2003). In extreme cases, complete loss of the signal can occur, as in Hawaiian field crickets (*Teleogryllus oceanicus*) that are parasitized by eavesdropping flies (*Ormia ochracea*) (Zuk et al. 2006). Avoiding the sensory range of natural enemies is not the only way to reduce detection, as signals could instead evolve to exploit perceptual errors of eavesdropping enemies. Here, we investigate this hypothesis and provide evidence of signalers exploiting the sensory systems of eavesdropping predators and parasites through an auditory illusion. Specifically, we examine how signal synchronization in pug-nosed treefrogs (*Smilisca sila*) can result in acoustic crypsis from such natural enemies.

When two sounds are closely synchronized, humans as well as other acoustically sensitive animals exhibit a perceptual localization bias towards the first, leading sound over the following sound (Wallach et al. 1949). Through this “precedence effect”, even when the sources are separated in space, the receiver perceives both sounds as having originated from the source of the leading sound. By producing signals closely synchronized with those of neighboring conspecifics, signaling males could create an illusion that obscures the locational information of their signals from receivers. Due to the precedence effect, females typically favor leading signals

over signals produced by following males, and as a result, males producing auditory displays typically avoid overlapping their mating signals (Greenfield et al. 1997; Gerhardt and Huber 2002). Synchronized signals, however, could also obscure the location of the following male from nontarget receivers. Despite the potential cost to females, male pug-nosed treefrogs produce calls in near-perfect synchrony with neighboring conspecifics (Figure 2.1a), overlapping call production at extremely short latencies (Ryan 1986). Males of this species, however, are also preyed upon by frog-eating bats (*Trachops cirrhosus*) and frog-biting midges (*Corethrella* spp.), both of which use calls to localize the frogs. Earlier work (Tuttle and Ryan 1982) revealed that eavesdropping bats and midges preferentially attack choruses of pug-nosed treefrogs calling artificially out-of-synchrony over synchronized choruses, as males naturally call in this species. It is unclear, however, how call order between individual males synchronizing their calls within the chorus affect receiver attraction. To examine this case of potential acoustic crypsis, we investigate how these bats and midges respond to the illusion generated by overlapping leading and following signals. Ultimately, we seek to determine whether these predators experience a precedence effect with synchronized calls. We predict that males producing following calls exploit the precedence effect in their eavesdropping enemies, reducing their attraction of both non-target receivers compared to the calls of leading males. It is puzzling, however, how such signal crypsis could evolve if it also comes at a cost to mate attraction. Therefore, we also investigate how target receivers, conspecific females, respond to synchronization.

2.3 Methods

Following previously established methods (bats: Page and Ryan 2008; midges: Bernal et al. 2006; frogs: Dyson and Passmore 1988) we conducted a series of both laboratory and field acoustic playback experiments to assess the leader-follower call preferences in the target receiver (female frogs) and non-target eavesdroppers (bats and midges). In addition, we established a reference for the strength or weakness of the precedence effect in female pug-nosed treefrogs, by also assessing leader-follower preferences of female frogs from a sympatric anuran species in which males do not synchronize their calls, the túngara frog (*Engystomops pustulosus*). In total, we performed four independent phonotaxis experiments: three testing bat, midge, and female pug-nosed treefrog preferences broadcasting male pug-nosed treefrog calls, and one experiment testing female túngara frog preferences using male túngara frog calls (Figure 2.1b). All

experiments were conducted in and around Gamboa, Republic of Panama (9°07.0'N, 79°41.9'W). For each experiment, a pair of speakers broadcast synchronized recordings of male frog calls with 79 ms of latency (average for pug-nosed treefrogs, Ryan 1986). Within a trial, one speaker out of the set always broadcast leading calls and the other speaker always broadcast following calls. Which of the two speakers broadcast which treatment, leading or following, was randomized for each trial to account for any side biases in receiver attraction. The speakers were placed at a distance that roughly matched the spacing between neighboring males in a chorus of either species (see Video 1 for examples of receiver phonotaxis recorded during the experiments).

A sample of ten recorded calls, each from different males, were used to build a stimulus library of 90 different leader-follower combinations. In this way, two libraries were built, one for synchronized pug-nosed treefrog calls and one for synchronized túngara frog calls. For each trial, stimuli were randomly drawn from the respective library. Only one stimuli, one set of synchronized calls, was used per trial. For the pug-nosed treefrog stimuli, only recordings of non-overlapped calls were used to avoid any prior artifacts from acoustic interference. Recordings were edited using CoolEdit2000 (Syntrillium Software), and stimuli were broadcast at a rate of one call every 2 s, at an amplitude of 75 dB SPL re. 20 μ P at 1 m from the speakers measured at ground level.

2.3.1 Frog-eating bat preference

Ten wild-caught bats were captured from June 1st to August 16th, 2017 using mist nets set along streams and small ponds. Each bat was marked with a passive transponder (PIT tags, Trovan, Ltd.), and tested individually in a 5x5x2.5m outdoor flight cage between 1900 and 0200h. During experiments, the bats were presented with two Fostex speakers (Model FE103En; Foster Electric Company, Tokyo, Japan) baited with fish (following Page and Ryan 2006; Hemingway et al. 2018) and spaced 1.5 m apart, broadcasting leading and following pug-nosed treefrog calls. Each bat was repeatedly tested using a new set of two recordings for a total of 10 consecutive choices (N = 100 choices). A choice was defined by flight within 50cm of a speaker (Hemingway et al. 2018). The number of times out of the 10 trials that a bat chose either leading

or following calls was recorded. The bats were returned to their initial capture location following experiments.

2.3.2 Frog-biting midge preference

Frog-biting midges were collected from January 11th to March 5th, 2017 using acoustic insect traps (McKeever and Hartberg 1980) placed over two pignose portable amplifier speakers (Model 7-100; Pignose-Gorilla, North Las Vegas, Nevada) spaced 1 m apart. Collection sites were randomly chosen in the forest within 100 m of a water source with breeding pug-nosed tree frogs. The set of speakers broadcast leading and following pug-nosed treefrog calls, randomly drawn from the stimulus library. The two speakers with their corresponding traps were run once per night for 45 min following sunset. A total of 1095 frog-biting midges were collected over a sampling period of 21 nights. The number of midges attracted each night to either the leading or following calls was used as an indicator of preference. Following their collection, frog-biting midges were counted and preserved in 75% ethanol for identification to species. A representative sample of the specimens was mounted and deposited in the collections at Purdue University.

2.3.3 Female frog preference

Phonotaxis experiments using female pug-nosed treefrogs ($n = 23$) were conducted from February 2nd to March 4th, 2017. Pairs of frogs were collected in amplexus from natural occurring choruses and transported to a 2m×3m semi-anechoic chamber (ETS-Lindgren, Cedar Park, TX). Females found in amplexus were used because they are highly receptive to male calls (Lynch *et al.* 2005). Immediately before testing, females were separated from the males and placed in the chamber under an acoustically transparent cup 1.5 m from a set pignose speakers spaced 1 m apart broadcasting leading and following pug-nosed treefrog calls. Females were kept under the cup for 1 min to adjust to the chamber before being released. Following standard rules used in phonotaxis experiments with anurans (Ryan and Rand 1990), a choice for either the leading or following call was scored after the female approached within 10 cm of a speaker. Females were tested once and released with their males at the end of the night. This experiment was repeated from October 17th to 25th, 2017, using female túngara frogs ($n = 40$) and broadcasts of synchronized túngara frog calls. Although leader-follower preferences have previously been

assessed in túngara frogs (Schwartz and Rand 1991; unpublished data discussed in Greenfield and Rand 2000), we aimed to test túngara frogs using an experimental design that mirrored the methods used here for pug-nosed treefrogs.

2.3.4 Statistical analyses

All analyses were conducted in R v. 3.5.2 (R Development Core Team 2015). Bat and midge preferences were independently analyzed using a two-tailed exact symmetry tests in the *coin* R package (Hothorn et al. 2008), testing the null hypothesis that preference was even between the leading and following calls. For the bat experiment, the number of choices for leading and following calls were compared, with the repeated measurements blocked within each individual bat ($N = 100$ choices, $n = 10$ choices/individual). For the midge preference experiment, the number of midges attracted to the leading and following calls was compared, blocked within each night ($n = 21$ nights). For both female preference experiments, pug-nose treefrog ($n = 23$ frogs) and túngara frog ($n = 40$ frogs), choice for either leading or following calls was analyzed using a two-tailed exact binomial choice test, again testing the null hypothesis that preference was even between the leading and following calls. Additionally, given the smaller sample-size for the female pug-nose treefrog experiment, concerns about a potential Type II error were excluded using a statistical power analysis using G*Power 3.1 (Faul et al. 2009). Data underlying these statistical analyses have been deposited in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.8w9ghx3gs> (Legett et al. 2019).

2.4 Results

Both bats and midges, the non-target eavesdropping receivers, had a significant preference for leading pug-nosed treefrog calls over following calls. Frog-eating bats chose the speaker broadcasting leading calls on average 70% of the time ($Z = 2.65$, $p = 0.004$; Figure 2.2a). Leading speakers also attracted an average of 55% more midges per night than speakers broadcasting following calls (31.67 ± 11.60 midges for leading calls vs. 20.48 ± 5.05 midges for following calls; $Z = 2.66$, $p = 0.005$; Figure 2.2b).

Female pug-nosed treefrogs, the target receivers, displayed no significant preference for leading or following calls, with only 15 of the 23 females tested choosing the leading call ($p = 0.21$; Figure 2.2c). In contrast, female túngara frogs, a non-synchronous species, displayed a strong preference with 36 of the 40 females choosing leading conspecific calls ($p < 0.001$; Figure 2.2d).

Using the effect size of the female túngara frog choice experiment for comparison, the female pug-nosed treefrog preference experiment had adequate statistical power ($1-\beta = 0.99$, $\alpha = 0.05$, effect size = 0.40). In addition to female túngara frogs, a similar level of leading call preference has been found in other anuran species (effect size = 0.35 ± 0.06 calculated from Schwartz 1986; Dyson and Passmore 1988; Grafe 1996; Bosch and Márquez 2002; Marshall and Gerhardt 2010; Reichert et al. 2016). If female pug-nosed treefrogs had leader-follower preferences comparable to female túngara frogs and other anuran species tested to date, we would expect 20 or 21 of the 23 tested female pug-nosed treefrog to prefer leading calls.

2.5 Discussion

Our results from the bat and midge preference experiments suggest that both predatory eavesdroppers are vulnerable to the precedence effect illusion and thus susceptible to exploitation. For acoustic receivers in general, the precedence effect is thought to be an adaptation for successfully localizing sounds in structurally complex environments, where reflections and reverberations carry spatial cues unrelated to the source of the signal (Litovsky et al. 1999). In these acoustic situations, the auditory system gives perceptual dominance of spatial information to the first arriving signal. Both the eavesdropping bats and midges forage in dense forest habitats, relying on their ability to detect and localize frog calls to find their prey. Therefore, despite drastic differences between perceptual systems, it is not unexpected that these two receivers experience this auditory effect.

Similar to the acoustic challenges facing the predators, female frogs must also localize calls in structurally complex environments to find mates and are therefore also expected to experience a precedence effect. This prediction is confirmed here in the strong leading call preference displayed by female túngara frogs and is characteristic of the response of females from most anuran species examined to date (e.g. Schwartz 1986; Dyson and Passmore 1988; Grafe 1996;

Bosch and Márquez 2002; Marshall and Gerhardt 2010; Reichert et al. 2016). For male frogs of these species, benefits from exploiting the precedence effect in eavesdroppers by synchronizing are not balanced out by the substantial cost imposed on the male producing the less attractive following calls. Female pug-nosed treefrogs, in contrast, break from this expected bias for leading signals suggesting that they do not experience a precedence effect, or that the strength of this effect is perceptually reduced. Thus, unlike túngara frogs, male pug-nosed treefrogs producing following calls benefit from reduced eavesdropper attraction without suffering the cost of reduced female attraction. Why then, would a male pug-nosed treefrog produce leading calls? With the reduced cost to producing following calls, but higher cost of producing leading calls, males compete in a “war of attrition” game (Grafe 2003), in which they try to avoid being the first to call. Eventually, however, males must produce calls to attract mates. Thus, males that produce the first, leading calls “lose” this intrasexual competition. As a result, pug-nosed treefrog choruses are characterized by long periods of silence punctuated by short, sporadic bouts of synchronized calls (Tuttle and Ryan 1982). Whether certain males within the chorus always end up leading in this game is currently unknown. Recent advances in sound-visualization techniques (Mizumoto et al. 2011) may help investigate the signaling interactions between multiple synchronizing males.

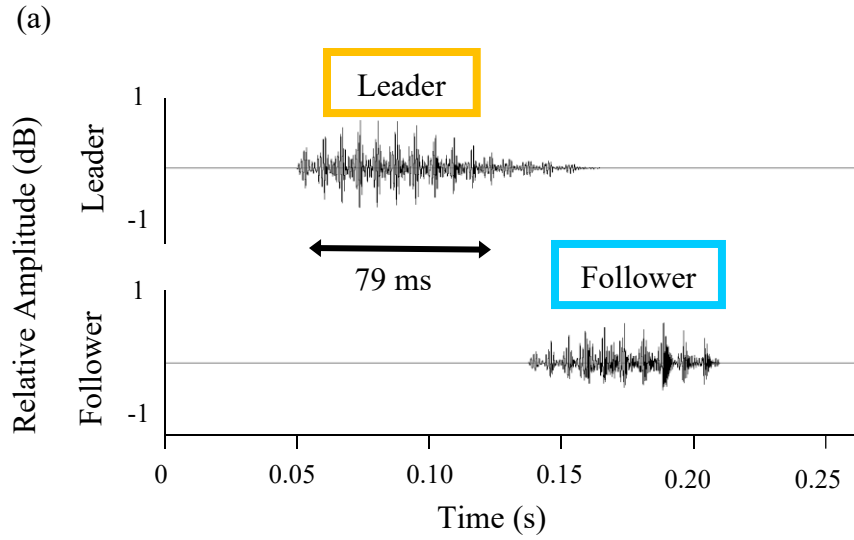
In addition to female pug-nosed treefrogs, atypical or reversed leader-follower preferences have been observed in other synchronizing anuran species (*Dendropsophus ebraccatus*: Wells and Schwartz 1984; *Kassina fusca*: Grafe 1999). Therefore, a loss of this preference, overcoming the precedence effect illusion, may be key to the evolution and maintenance of this signaling strategy. How females of synchronizing species are able to overcome the precedence effect is still a mystery. Studies that examine the auditory physiology, specifically temporal processing, of such species and their non-synchronous congeners would provide insight into the mechanisms underlying the use of synchronized mating signals. Overall, obtaining a thorough understanding of the evolutionary drivers shaping signals and signaling strategies requires understanding of the individual and combined selective pressures imposed by target and non-target receivers. A communication network approach (McGregor and Peake 2000) examining selective pressure from multiple receivers, as the one used here, provides important insights to understand these processes shaping signal evolution. Further studies that provide a phylogenetic framework to

understanding the sequence in which costs and benefits from multiple receivers unfolded is also necessary to better understand the evolution of signals.

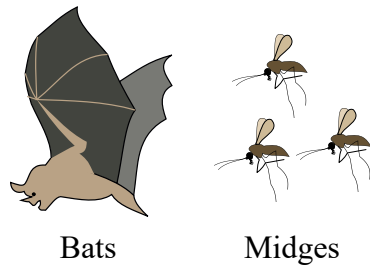
Finally, while this study focuses on the male vocal behavior of synchronization, the costs and benefits to overcoming the precedence effect for the female receiver warrants corresponding consideration. By not experiencing a precedence effect, females may be able to discriminate between preferred and non-preferred males independent of call timing (Tárano 2015; Schwartz and Serratto Del Monte 2018). In addition, since female frogs can avoid approaching males associated with higher perceive predation risk (Bonachea and Ryan 2011), and males with leading calls are more attractive to predators, overcoming the precedence effect may allow females to reduce their risk of predation. Further studies from the female's perspective, in particular examining the predation rates associated with different mate choices, are needed to better understand the tradeoffs of signal synchronization, and other signaling strategies.

We show that by synchronizing their calls with those of neighbor males, pug-nosed treefrogs exploit the sensory perspective of eavesdropping enemies yielding less attacks. Thus, our results suggest that misjudgments in sensory systems of predators can play a direct role in shaping mate signaling strategies. While prey manipulation of predators' sensory systems to misdirect or reduce detection has long been acknowledged (Kelley and Kelley 2013; Rubin et al. 2018), this study provides the first case of perceptual manipulation of eavesdroppers through illusions of mating signals. Mating signals are at the intersection of natural and sexual selection, driven to increase attraction of target receivers but simultaneously avoid the attraction of non-targets.

Therefore, as perceptual exploitation of mates by signaling males has proven valuable to understanding signal evolution (Endler and Basolo 1998; Ryan and Cummings 2013), exploitations of eavesdropping predators are also likely to provide valuable insights. Ultimately, we show that auditory illusions can be exploited to decrease predation without decreasing mate attraction. Similar sensory manipulations of predatory eavesdroppers are expected in other organisms, and across sensory modalities beyond the acoustic realm.



(b) Non-Target Receivers



Target Receivers

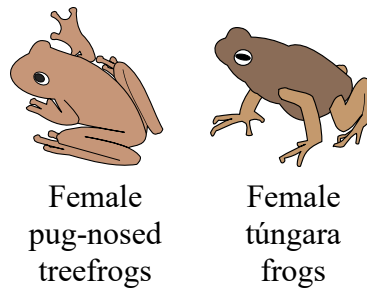


Figure 2.1. Oscillograms (a) of leading and following calls of two male pug-nosed treefrogs, and (b) the target and non-target call receivers included in this study. The calls are synchronized with 79 ms of latency, the observed natural average in this species (Ryan 1986).

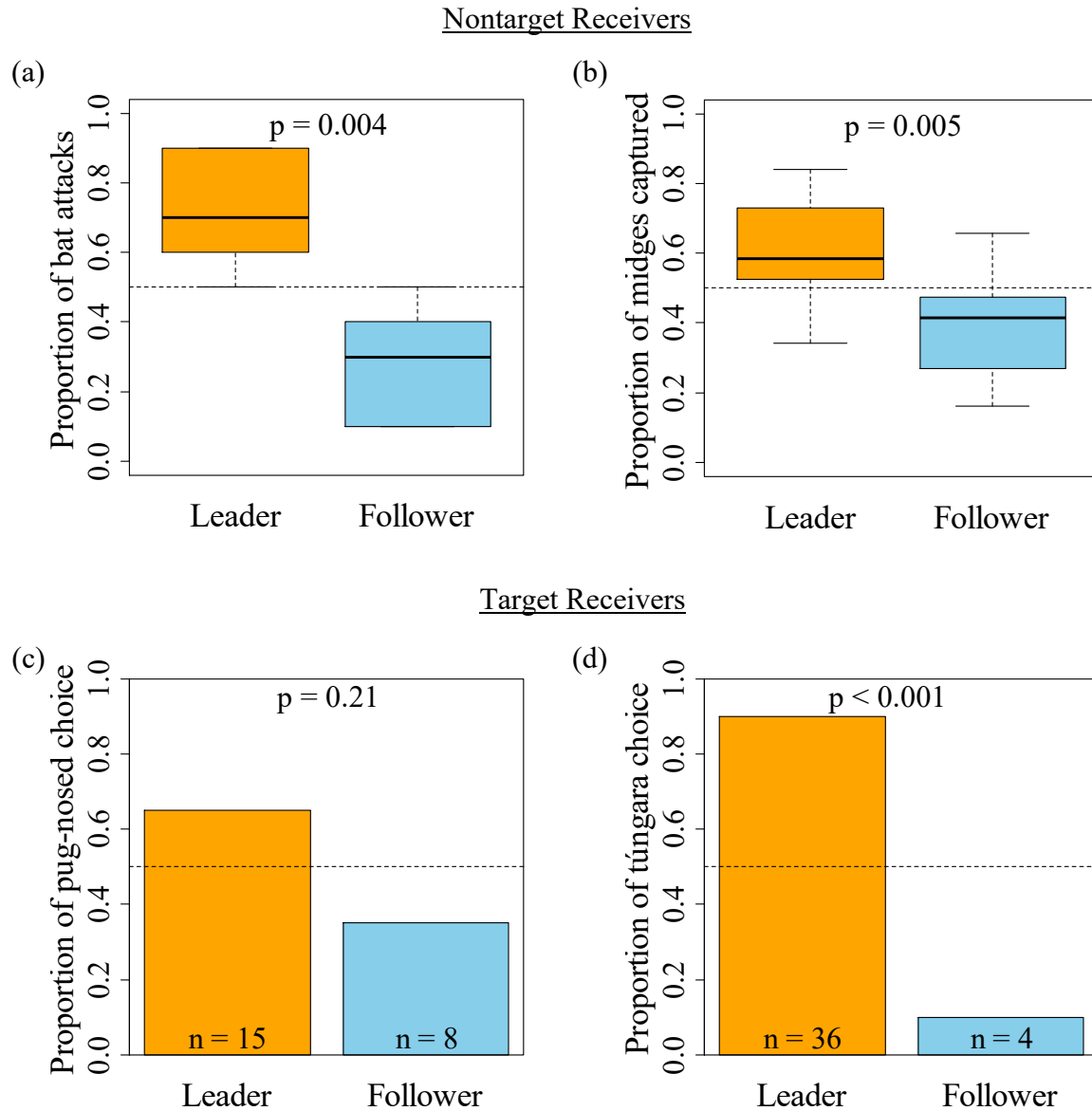


Figure 2.2. The proportion of non-target (a bats and b midges) and target (c female pug-nosed frogs) receivers that selected for leading or following recorded calls of male pug-nosed treefrogs. The preferences of another target receiver, female túngara frogs (d), were assessed using male túngara frog calls.

2.6 References

- Bernal, X. E., Rand, A. S., and Ryan, M. J. (2006). Acoustic preferences and localization performance of blood-sucking flies (*Corethrella* Coquillett) to túngara frog calls. *Behavioral Ecology* 17: 709-715.
- Bonachea, L. A., and Ryan, M. J. (2011). Simulated predation risk influences female choice in túngara frogs, *Physalaemus pustulosus*. *Ethology* 117: 400-407.
- Bosch, J., and Márquez, R. (2002). Female preference function related to precedence effect in an amphibian anuran (*Alytes cisternasii*): tests with non-overlapping calls. *Behavioral Ecology* 13: 149-153.
- Christy, J. H. (1995). Mimicry, mate choice, and the sensory trap hypothesis. *The American Naturalist* 146: 171-181.
- Cummings, M. E., Rosenthal, G. G., and Ryan, M. J. (2003). A private ultraviolet channel in visual communication. *Proceedings of the Royal Society B* 270: 897-904.
- Dyson, M. L., and Passmore, N. I. (1988). Two-choice phonotaxis in *Hyperolius marmoratus* (Anura: Hyperoliidae): the effect of temporal variation in presented stimuli. *Animal Behaviour* 36: 648-652.
- Endler, J. A., and Basolo, A. L. (1998). Sensory ecology, receiver biases and sexual selection. *Trends in Ecology and Evolution* 13: 415-420.
- Faul, F., Erdfelder, E., Buchner, A., and Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods* 41: 1149-1160.
- Gerhardt, H. C., and Huber, F. (2002). *Acoustic communication in insects and anurans: common problems and diverse solutions*. University of Chicago Press, Chicago.
- Grafe, T. U. (1996). The function of call alternation in the African reed frog (*Hyperolius marmoratus*): precise call timing prevents auditory masking. *Behavioral Ecology and Sociobiology* 38: 149-158.
- Grafe, T. U. (1999). A function of synchronous chorusing and a novel female preference shift in an anuran. *Proceedings of the Royal Society B* 266: 2331-2336.
- Grafe, T. U. (2003). Synchronized interdigitated calling in the Kuvangu running frog, *Kassina kuvangensis*. *Animal Behaviour* 66: 127-136.
- Greenfield, M. D., and Rand, A. S. (2000). Frogs have rules: selective attention algorithms regulate chorusing in *Physalaemus pustulosus* (Leptodactylidae). *Ethology* 106: 331-347.
- Greenfield, M. D., Tourtellot, M. K., and Snedden, W. A. (1997). Precedence effects and the evolution of chorusing. *Proceedings of the Royal Society B* 264: 1355-1361.
- Hemingway, C. T., Ryan, M. J., and Page, R. A. (2018). Cognitive constraints on optimal foraging in frog-eating bats. *Animal Behaviour* 143: 43-50.
- Hothorn, T., Hornik, K., van de Wiel, M. A., and Zeileis, A. (2006). Implementing a Class of Permutation Tests: The *coin* Package. *Journal of Statistical Software* 28: 1-23.
- Kelley, L. A., and Kelley, J. L. (2013). Animal visual illusion and confusion: the importance of a perceptual perspective. *Behavioral Ecology* 25: 450-463.
- Legett, H. D., Hemingway, C. T., and Bernal, X. E. (2019). Data from: Prey exploits the auditory illusions of eavesdropping predators. *The American Naturalist*, Dryad Digital Repository, <https://doi.org/10.5061/dryad.8w9ghx3gs>.
- Litovsky, R. Y., Colburn, H. S., Yost, W. A., and Guzman, S. J. (1999). The precedence effect. *The Journal of the Acoustical Society of America* 106: 1633-1654.

- Lynch, K. S., Rand, A. S., Ryan, M. J., and Wilczynski, W. (2005). Plasticity in female mate choice associated with changing reproductive states. *Animal Behaviour* 69: 689-699.
- Marshall, V. T., and Gerhardt, H. C. (2010). A precedence effect underlies preferences for calls with leading pulses in the grey treefrog, *Hyla versicolor*. *Animal Behaviour* 80: 139-145.
- McGregor, P. K., and Peake, T. M. (2000). Communication networks: social environments for receiving and signalling behaviour. *Acta Ethologica* 2: 71-81.
- McKeever, S., and Hartberg, W. K. (1980). An effective method for trapping adult female *Corethrella* (Diptera: Chaoboridae). *Mosquito News* 20: 111-112.
- Mizumoto, T., Aihara, I., Otsuka, T., Takeda, R., Aihara, K., and Okuno, H. G. (2011). Sound imaging of nocturnal animal calls in their natural habitat. *Journal of Comparative Physiology A* 197: 915-921.
- Page, R. A., and Ryan M. J. (2006). Social transmission of novel foraging behavior in bats: frog calls and their referents. *Current Biology* 16: 1201-1205.
- Page, R. A., and Ryan, M. J. (2008). The effect of signal complexity on localization performance in bats that localize frog calls. *Animal Behaviour* 76: 761-769.
- R Development Core Team. (2015). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Reichert, M. S., Symes, L. B., and Höbel, G. (2016). Lighting up sound preferences: cross-modal influences on the precedence effect in treefrogs. *Animal Behaviour* 119: 151-159.
- Rubin, J. J., Hamilton, C. A., McClure, C. J., Chadwell, B. A., Kawahara, A. Y., and Barber J. R. (2018). The evolution of anti-bat sensory illusions in moths. *Science Advances* 4: eaar7428.
- Ryan, M. J. (1986). Synchronized calling in a treefrog (*Smilisca sila*). *Brain, Behavior and Evolution* 29: 196-206.
- Ryan, M. J. (1990). Sexual selection, sensory systems and sensory exploitation. *Oxford Surveys in Evolutionary Biology* 7: 157-195.
- Ryan, M. J., and Rand, A. S. (1990). The sensory basis of sexual selection for complex calls in the túngara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). *Evolution* 44: 305-314.
- Ryan, M. J., and Cummings, M. E. (2013). Perceptual biases and mate choice. *Annual Review of Ecology, Evolution, and Systematics* 44: 437-459.
- Schwartz, J. J. (1986). Male calling behavior and female choice in the neotropical treefrog *Hyla microcephala*. *Ethology* 73: 116-127.
- Schwartz, J. J., and Rand, A. S. (1991). The consequences for communication of call overlap in the Túngara frog, a neotropical anuran with a frequency-modulated call. *Ethology* 89: 73-83.
- Schwartz, J. J., and Serratto Del Monte, M. E. (2018). Spatially-mediated call pattern recognition and the cocktail party problem in treefrog choruses: can call frequency differences help during signal overlap?. *Bioacoustics* 28: 312-328.
- Tárano, Z. (2015). Choosing a mate in a cocktail party-like situation: the effect of call complexity and call timing between two rival males on female mating preferences in the túngara frog *Physalaemus pustulosus*. *Ethology* 121: 749-759.
- Tuttle, M. D., and Ryan, M. J. (1982). The role of synchronized calling, ambient light, and ambient noise, in anti-bat-predator behavior of a treefrog. *Behavioral Ecology and Sociobiology* 11: 125-131.

- Wallach, H., Newman, E. B., and Rosenzweig, M. R. (1949). A precedence effect in sound localization. *The Journal of the Acoustical Society of America* 21: 468-468.
- Wells, K. D., and Schwartz, J. J. (1984). Vocal communication in a neotropical treefrog, *Hyla ebraccata*: aggressive calls. *Behaviour* 91: 128-145.
- Zuk, M., and Kolluru, G. R. (1998). Exploitation of sexual signals by predators and parasitoids. *The Quarterly Review of Biology* 73: 415-438.
- Zuk, M., Rotenberry, J. T., and Tinghitella, R. M. (2006). Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biology Letters* 2: 521-524.

CHAPTER 3. SIGNAL SYNCHRONY AND ALTERNATION AMONG NEIGHBORING MALES IN A JAPANESE STREAM BREEDING TREEFROG, *BUERGERIA JAPONICA*

H. D. Legett¹, I. Aihara², X. E. Bernal^{1,3}

¹ *Purdue University, West Lafayette, IN, USA*

² *University of Tsukuba, Tsukuba, Ibaraki, JP*

³ *Smithsonian Tropical Research Institute, Balboa, Ancón, Panama, PA*

Citation

This chapter was originally published in the journal *Current Herpetology* and is reproduced here with permission.

Legett, H. D., Aihara, I., and Bernal, X. E. (2020). Signal Synchrony and Alternation Among Neighbor Males in a Japanese Stream Breeding Treefrog, *Buergeria japonica*. *Current Herpetology* 39: 80-85.

3.1 Abstract

Animals that aggregate in leks to attract mates often time the production of their mating signals against the signals of neighboring conspecific. Such signal timing usually falls into general patterns within these aggregations, which can be categorized based on the amount of overlap between the signals. Many species produce signals in an alternating pattern, avoiding signal overlap to reduce interference and increase mate attraction. In contrast, 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.

3.2 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 granulose*: Ibáñez 1993; *Kassina fusca*: Grafe 1999; *Kassinia kuvagenus*: 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 japonica*), 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 (Figure 3.1a). 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 (Figure 3.1b). 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*.

3.3 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 μ P (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 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.

3.4 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 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 ($\pm$ standard error) after the playback had ended (Figure 3.2a), 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 % (Figure 3.2b). 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 μ P 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 (Tuttle and Ryan 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 (Johnsons et al. 1993; Bernal and Pinto 2016). Male *B. japonica* may synchronize 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.

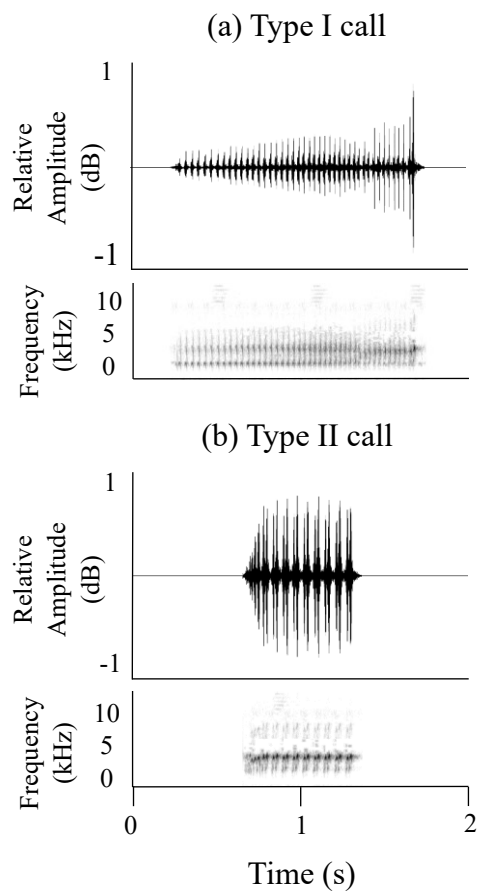


Figure 3.1. Oscillograms and spectrograms of Type I (a) and Type II (b) mating calls.

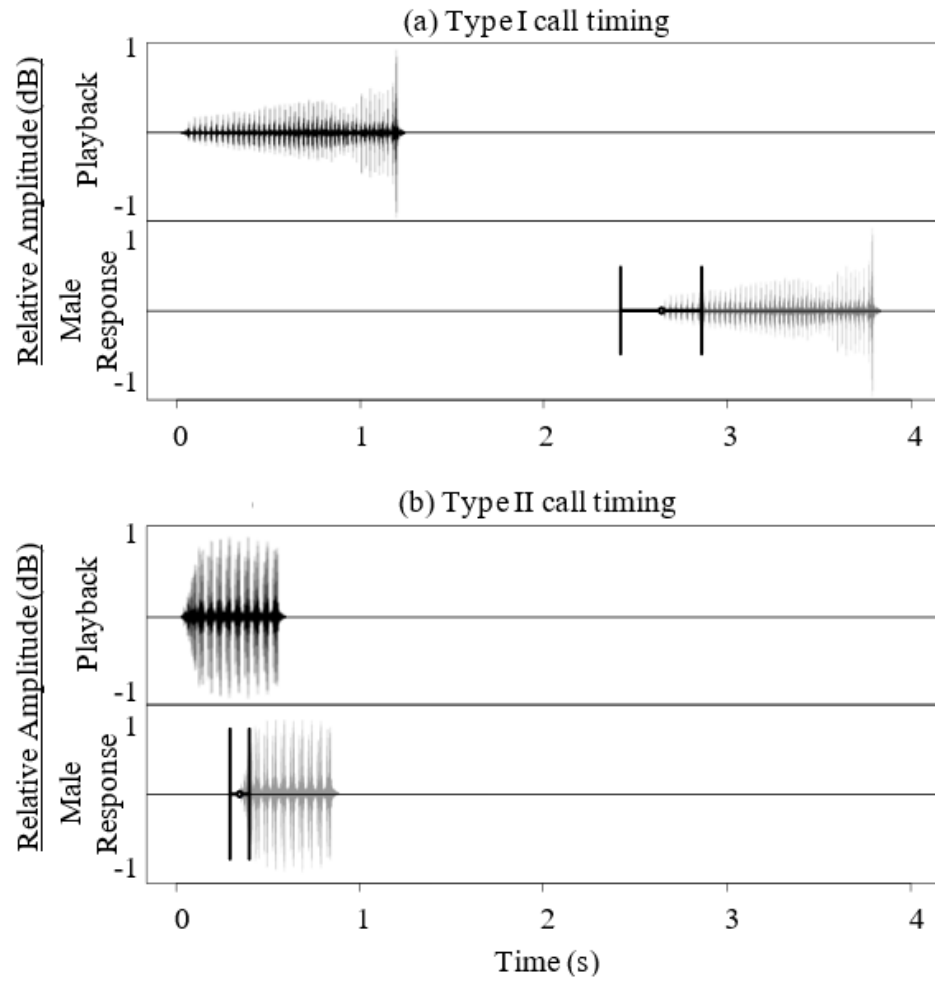


Figure 3.2. Average timing of male responses to Type I (a) and Type II (b) call playbacks. Bars show standard error.

3.5 References

- Bee, M. A., and Micheyl, C. (2008). The cocktail party problem: what is it? How can it be solved? And why should animal behaviorists study it?. *Journal of Comparative Psychology* 122: 235-251.
- Bernal, X. E., and Pinto, C. M. (2016). Sexual differences in prevalence of a new species of trypanosome infecting túngara frogs. *International Journal for Parasitology: Parasites and Wildlife* 5: 40-47.
- Brumm, H., and Slabbekoorn, H. (2005). Acoustic communication in noise. *Advances in the Study of Behavior* 35: 151-209.
- Camp, J. V. (2006). *Host attraction and host selection in the family Corethrellidae (Wood and Borkent)(Diptera)*. Unpublished master's thesis. Georgia Southern University, Statesboro.
- Capshaw, G., Foss-Grant, A. P., Hartmann, K., Sehuanes, J. F., and MOSSoss, C. F. (2018). Timing of the advertisement call of the common tink frog (*Diasporus diastema*) shifts with the acoustic behaviour of local conspecifics. *Bioacoustics* 1-18.
- Clulow, S., Mahony, M., Elliott, L., Humfeld, S., and Gerhardt, H. C. (2017). Near-synchronous calling in the hip-pocket frog *Assa darlingtoni*. *Bioacoustics* 26:249-258.
- Farabaugh, S. M. (1982). The ecological and social significance of duetting. *Acoustic communication in birds* 2: 85-124.
- Gerhardt, H. C., and Huber, F. (2002). *Acoustic communication in insects and anurans: common problems and diverse solutions*. University of Chicago Press, Chicago.
- Gerhardt, H. C., and Klump, G. M. (1988). Masking of acoustic signals by the chorus background noise in the green tree frog: a limitation on mate choice. *Animal Behaviour* 36: 1247-1249.
- Grafe, T. U. (1999). A function of synchronous chorusing and a novel female preference shift in an anuran. *Proceedings of the Royal Society B* 266: 2331-2336.
- Grafe, T. U. (2003). Synchronized interdigitated calling in the Kuvangu running frog, *Kassina kuvangensis*. *Animal Behaviour* 66: 127-136.
- Greenfield, M. D. (1994). Cooperation and conflict in the evolution of signal interactions. *Annual Review of Ecology and Systematics* 25: 97-126.
- Ibáñez, R. (1993). Female phonotaxis and call overlap in the neotropical glassfrog *Centrolenella granulosa*. *Copeia* 1993: 846-850.
- Johnson, R. N., Young, D. G., and Butler, J. F. (1993). Trypanosome transmission by *Corethrella wirthi* (Diptera: Chaoboridae) to the green treefrog, *Hyla cinerea* (Anura: Hylidae). *Journal of Medical Entomology* 30: 918-921.
- Legett, H. D., Page, R. A., and Bernal, X. E. (2019). Synchronized mating signals in a communication network: the challenge of avoiding predators while attracting mates. *Proceedings of the Royal Society B* 286: 20191067.
- Kuramoto, M. (1986). Call structures of the rhacophorid frogs from Taiwan. *Scientific report of the Laboratory for Amphibian Biology* 8: 45-68.
- Mizumoto, T., Aihara, I., Otsuka, T., Takeda, R., Aihara, K., and Okuno, H. G. (2011). Sound imaging of nocturnal animal calls in their natural habitat. *Journal of Comparative Physiology A* 197: 915-921.
- Ryan, M. J. (1986). Synchronized calling in a treefrog (*Smilisca sila*). *Brain, Behavior and Evolution* 29: 196-206.
- Ryan, M. J., Tuttle, M. D., and Rand, A. S. (1982). Bat predation and sexual advertisement in a neotropical anuran. *The American Naturalist* 119: 136-139.

- Shelly, T. E., and Greenfield, M. D. (1991). Dominions and desert clickers (Orthoptera: Acrididae): influences of resources and male signaling on female settlement patterns. *Behavioral Ecology and Sociobiology* 28: 133-140.
- Tang, W. (2009). *The chorus sound characteristics and phonotactic behavior of Buergeria japonica*. Unpublished master's thesis. National Cheng Kung University, Tainan City.
- Toma, T., Miyagi, I., and Tamashiro, M. (2014). Blood meal identification and feeding habits of *Uranotaenia* species collected in the Ryukyu Archipelago. *Journal of the American Mosquito Control Association* 30: 215-219.
- Tuttle, M. D., and Ryan, M. J. (1982). The role of synchronized calling, ambient light, and ambient noise, in anti-bat-predator behavior of a treefrog. *Behavioral Ecology and Sociobiology* 11: 125-131.
- Wang, Y. H., Hsiao, Y. W., Lee, K. H., Tseng, H. Y., Lin, Y. P., Komaki, S., and Lin, S. M. (2017). Acoustic differentiation and behavioral response reveals cryptic species within *Buergeria* treefrogs (Anura, Rhacophoridae) from Taiwan. *PloS one* 12: e0184005.
- Wells, K. D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour* 25: 666-693.
- Wells, K. D., and Schwartz, J. J. (1984). Vocal communication in a neotropical treefrog, *Hyla ebraccata*: advertisement calls. *Animal Behaviour* 32: 405-420.
- Wells, K. D., and Schwartz, J. J. (2007). The behavioral ecology of anuran communication. pp. 44-86. In: *Hearing and sound communication in amphibians*. Springer, New York.
- Wollerman, L. (1999). Acoustic interference limits call detection in a Neotropical frog *Hyla ebraccata*. *Animal Behaviour* 57: 529-536.

CHAPTER 4. WITHIN HOST ACOUSTIC SIGNAL PREFERENCE OF FROG-BITING MOSQUITOES (DIPTERA: CULICIDAE) AND MIDGES (DIPTERA: CORETHRELLIDAE) ON IRIOMOTE ISLAND, JAPAN

H. D. Legett¹, I. Aihara², X. E. Bernal^{1,3}

¹ *Purdue University, West Lafayette, IN, USA*

² *University of Tsukuba, Tsukuba, Ibaraki, JP*

³ *Smithsonian Tropical Research Institute, Balboa, Ancón, Panama, PA*

4.1 Abstract

Haematophagous insects can rely on specialized host-seeking behaviors to locate hosts. Some frog-biting flies, for example, eavesdrop on the conspicuous acoustic signals produced by male frogs and toads. Using such auditory cues to locate a host imposes an additional challenge: how to recognize appropriate sounds when different frog species produce calls with varying acoustic properties. The limited knowledge of antennal hearing hinders our ability to understand how eavesdropping flies detect and recognize frog calls. Behavior studies suggest that frog-biting flies use broad acoustic templates and are preferentially attracted to lower frequency calls. Here, we use within-species call variation to test this low-frequency bias hypothesis in frog-biting flies. Specifically, we examine the attraction of frog-biting mosquitoes (*Uranotaenia* spp.) and midges (*Corethrella nippon*) to the calls of a Japanese treefrog, the Ryukyu Kajika frog (*Buergeria japonica*), on Iriomote Island, Japan. Male Ryukyu Kajika frogs produce two call types. The first call type (Type I) contains lower spectral frequency elements compared to the second call type (Type II). Using field phonotaxis experiments we found that, as predicted, Type I calls were significantly more attractive to both frog-biting mosquitoes and midges. Thus, our results confirm the previously assumed sensory bias in species that exploit anuran calls and suggest this low-frequency bias may be general across frog-biting Nematoceran flies. We discuss this finding in the context of the evolution of antennal hearing in flies.

4.2 Introduction

Haematophagous insects rely on blood meals from hosts for reproduction and, in some cases, survival (Lehane 2005). There is thus strong selective pressure to locate appropriate hosts, which can result in specialized host-seeking behaviors involving a variety of sensory cues (Takken and Verhulst 2013). For example, some flies that bite frogs and toads, including mosquitoes of the genus *Uranotaenia* (Diptera: Culicidae) and midges of the genus *Corethrella* (Diptera: Corethrellidae), use auditory cues to locate their hosts (e.g. McKeever 1977; Borkent and Belton 2006; Bernal et al. 2006). These frog-biting flies eavesdrop on the conspicuous advertisement calls produced by male frogs and toads. However, using auditory cues to locate hosts can be challenging. Unlike other host recognition cues, such as temperature and carbon dioxide, anuran mating calls are species specific with high diversity of spectral and temporal acoustic properties (Duellman and Trueb 1986).

Behavioral studies suggest that at least some species of frog-biting mosquitoes and midges have generalized acoustic templates that allow them to detect and localize the calls of multiple anuran host species (Virgo et al. 2019; Toma et al. 2019; but see de Silva et al. in press). In some systems, frog-biting midges are attracted to a broad range of pure tones, 100–4000 Hz (Meuche et al. 2016). The ability to detect a broad range of frequencies can be beneficial for eavesdroppers as the abundance of specific hosts species can fluctuate across habitats and seasons (Legett et al. 2018). However, while many eavesdroppers are attracted to the calls produced by multiple host species, not all calls are equally attractive to them. In general, frog-biting flies seem to prefer frog calls that are more acoustically complex, spectrally broadband, and lower frequency, resulting in different eavesdropper attraction rates among host species (Virgo et al. 2019) and among different call types within a given host (Bernal et al. 2006). Even though the auditory mechanisms and sound perception by eavesdropping frog-biting flies are not fully understood (Page et al. 2014), their phonotactic responses in the field suggest their hearing system is biased towards lower frequency host calls. Here, we test this hypothesis by conducting field phonotaxis experiments examining the responses of eavesdropping flies to call variation within a host species. In particular, we investigate the attraction of frog-biting mosquitoes and midges to different call types of a Japanese treefrog, the Ryukyu Kajika frog (*Buergeria japonica*).

Male Ryukyu Kajika frogs produce two distinct call types (Figure 4.1a,b). The social functions of either call type are unknown, although both seem involved in mate attraction (Tang 2009; Wang et al. 2017). Both call types are trills, containing notes repeated in a rapid redundant succession, and both are produced at variable durations, ranging from truncated calls of less than 0.5 s to longer calls over 3.0 s (Kuramoto 1986). The first call type (Type I), however, has low and high spectral frequency elements (dominant frequency at approximately 1.8 kHz and a co-dominant band at 3 kHz), while the second call type (Type II) has only a high frequency element (approximately 3 kHz). Given the difference in spectral frequency between the two frog call types, we predict that Type I calls are more attractive to eavesdropping flies than Type II calls.

4.3 Methods

4.3.1 Eavesdropper selection for call type

To assess midge and mosquito attraction to the different call types of the Ryukyu Kajika frog, frog-biting insects were collected from 27 June to 25 July 2015 at six locations around Iriomote Island, Okinawa Prefecture, Japan (24°17'33"N 123°51'43"E). All locations were greater than 1 km apart (following Legett et al. 2019) and chosen to cover a range of habitats in which Ryukyu Kajika frog choruses occur; two in undeveloped forest, two near rice paddy fields, and two along roadside ditches. Each of the six locations were sampled five times over the collection period for a total of 30 trials. Eavesdropping insects were collected using modified CDC mosquito traps (McKeever and Hartberg 1980) placed over speakers (Pignose Model 7-100) following well-established, standard procedures (e.g. Bernal et al. 2006). Within a location, two speakers with traps were placed 30 m apart. One speaker broadcast Type I calls while the other broadcast Type II calls. Broadcast calls were haphazardly chosen from a library of 10 pre-recorded calls of each type. All calls in this library were recorded from different male frogs. Both call types were broadcast at a rate of one call every 2 s at an amplitude of 80 dB SPL re. 20 μ P (Brüel and Kjær digital sound level meter Type 2250) measured from 1 m at ground level. While the natural call rate and amplitude of Type I and Type II calls can differ, our goal was to investigate only the effect of call structure.

At a location, insects were collected for 45 min starting 30 min after sunset (Legett et al. 2019). After collection, all insects were euthanized overnight in a freezer. The following day, all the insects were counted and stored in 75% ethanol for species identification. Established keys were used to identify eavesdroppers to species, *Uranotaenia* (Miyagi and Toma 2013) and *Corethrella* (Borkent 2008). Since some moth flies of the genus *Sycorax* (Family: Psychodidae) are known to eavesdrop on frog calls (Cutajar and Roley 2020), we also counted and separated moth flies in our collection.

4.3.2 Statistical Analysis

Statistical analyses were conducted using Program R v. 3.5.2 (R Development Core Team: www.R-project.org). The difference in attraction of insects to each Ryukyu Kajika frog call type was analyzed using the generalized linear mixed effect model (GLMM) function in the *glmmTMB* package (Brooks et al. 2017). Call type (I or II) was included as a fixed factor, location as a random factor and date as a random factor nested within location. In some cases, multiple trials were performed at different locations on the same night, so trial number was also included as a random factor nested within date to account for potential effects of sampling time over the course of each night. The specific playbacks used for each trial (playback ID) was included as an additional, non-nested, random factor.

For the three groups of frog-biting insects (mosquitoes, midges, and potentially moth flies), GLMMs with Poisson distributions and log link functions were used to compare eavesdropper attraction between call types. Zero-inflated models were used for the mosquito and midge models, and a non-zero-inflated model was used for the moth flies. Differences in number of individuals collected as bycatch, non-frog-biting insects, were analyzed using a GLMM with a negative binomial II distributions and a log link function. All models were fit based on AIC (following procedures in Bolker et al. 2009) and evaluated using the model diagnostic R package *DHARMA* (Hartig 2018). Assumptions of homoscedasticity were additionally tested using Levene's tests in the *car* package (Fox and Weisberg 2019). Differences in the least squares means obtained from the models were used as estimates of effect sizes.

4.4 Results

Frog-call traps broadcasting Ryukyu Kajika frog calls were successful in capturing frog-biting mosquitoes ($n = 100$), midges ($n = 74$), and moth flies ($n = 37$), as well as numerous non-frog-biting insects (Table 4.1). Four frog-biting mosquito species were identified, two from the subgenus *Pseudoficalibia* (*Ur. nivipleura*, *Ur. ohamai*) and two from the subgenus *Uranotaenia* (*Ur. annandalei*, *Ur. macfarlanei*). Of these, *Ur. macfarlanei* was the dominant species, representing 71% of the mosquitoes captured. Significantly more frog-biting mosquitoes were captured using Type I calls, which attracted 2.80 ± 0.85 (mean $\pm$ standard error) mosquitoes per 45 min trial compared to 0.57 ± 0.16 mosquitoes for Type II calls ($z(53) = 4.25$, $p < 0.001$, effect size = 1.66; Figure 4.2a). Similarly, Type I calls were also significantly more attractive to frog-biting midges, attracting 2.00 ± 1.06 midges per 45 min trial compared to 0.47 ± 0.22 midges for Type II calls ($z(53) = 2.80$, $p = 0.005$, effect size = 1.37; Figure 4.2b). All captured frog-biting midges were of the same species, *Corethrella nippon*. In contrast to frog-biting mosquitoes and midges, the number of moth flies captured did not significantly differ between call types ($z(53) = 1.14$, $p = 0.253$, effect size = 0.38; Figure 4.2c). There was also no significant difference in the number of non-frog-biting insects captured between call types ($z(53) = -0.90$, $p = 0.374$, effect size = -0.19; Figure 4.2d).

4.5 Discussion

We found that the two Ryukyu Kajika frog call types differ in their attraction of eavesdropping flies. As expected, the call type with lower frequency elements (Type I calls) attracted significantly more frog-biting mosquitoes (*Uranotaenia* spp.) and midges (*Corethrella nippon*). This finding is consistent with the preferences of frog-biting midges found in neotropical systems, where frog species with lower frequency calls (< 1 kHz) attract significantly more midges than frog species with higher frequency calls (Virgo et al. 2019). A similar pattern in preference among the calls of host species has also been shown with frog-biting mosquitoes in Japan (Toma et al. 2019). The most abundant frog species with a low frequency call was generally the most attractive to all eavesdroppers (*Leptodactylus savagei*: Virgo et al. 2019; *Fejervarya sakishimensis*: Toma et al. 2019). These studies, however, relied on inter-species variation in call structure. Thus, these previous studies potentially confound variation in call

acoustics with trade-offs associated with biting different species. Low frequency calls, for instance, are associated with species of larger size, a feature that could modulate host defensive strategies. Our results, revealing differences in eavesdropper attraction rates among call types within a host species, support the low-frequency bias hypothesis and rule out alternative explanations arising from differences among host species. These within host findings are consistent with frog-biting midge preferences for the calls of túngara frogs (*Engystomops pustulosus*), where complex call types with more energy at low frequencies are more attractive than simple call types (Bernal et al. 2006). Túngara frog complex calls and Ryukyu Kajika frog Type I calls share yet another feature, they both have multiple frequency peaks. In sum, the phonotactic behavior of frog-biting flies tested here and in other systems suggests their differential attractiveness to frog calls is due to generalized acoustic templates biasing lower frequency sounds and sounds with complex frequency spectrum.

Despite the independent evolutionary origins of eavesdropping on frog calls, frog-biting mosquitoes and midges share their preferences for the same call features. Given that flies from both lineages use antennal hearing, this preference likely reflects sensory biases of this hearing organ. Antennal hearing has received great attention in a mating context in mosquitoes (Gopfert and Robert 1999; Nadrowski et al. 2011; Albert and Kozlov 2016), but less is known about how these particle detection organs would detect frog calls (Page et al. 2014). It has long been assumed, for instance, that antennal hearing was restricted to sounds at close distances (Gopfert and Robert 2001), questioning how frog-biting mosquitoes and midges could hear and phonotax to frog calls from a distance. Recent studies, however, indicate that the antennae of mosquitoes can detect sound sources farther away than previously predicted (Menda et al. 2019). As the use of acoustic signals to find hosts may have evolved from using sound in a mating context (de Silva et al. 2015), additional adaptations that facilitate detecting frog calls are expected to have evolved in frog-biting mosquitoes and midges. Such adaptations in antennal hearing, however, would have evolved under limitations imposed by morphological and physiological constraints, potentially resulting in the biases highlighted in this study. Further investigations of the biomechanical properties and auditory physiology of antennae of frog-biting flies are needed to better understand why general acoustic preferences seem to hold across eavesdropping frog-biting mosquito and midge species.

In contrast to frog-biting mosquitoes and midges, the moth flies captured in our study did not display a bias for either call type. The number of individual moth flies captured is comparable to those of other frog-biting species collected here, which may suggest the use of acoustic cues rather than random bycatch. A previous collection using acoustic frog call traps on Iriomote Island reported capturing moth flies at rates similar to those found in this study (Toma et al. 2005). However, that study found no difference between traps with frog calls and silent, control traps suggesting that moth flies in this system are not eavesdropping. Frog-biting moth flies from the genus *Sycorax* eavesdrop on frog calls in Australia (Cutajar and Rowley 2020), but species from this genus in other parts of the world do not seem to rely on host-emitted calls to bite frogs (Bravo and Salazar-Valenzuela 2009, Macat et al 2015). The prevalence of eavesdropping in other moth fly genera is unknown.

Finally, attracting eavesdropping flies can be costly for hosts due to blood loss (Camp 2006) and disease transmission (Johnson et al. 1993; Bernal and Pinto 2016). Thus, differential attraction of eavesdropping frog-biting insects is likely a driver of signal evolution among frogs and toads. Call variation and eavesdropper avoidance behaviors, in particular, can be impacted by selective pressures imposed by eavesdroppers (Legett et al. 2019). Examples of such avoidance behaviors in frogs include calling in habitats with high levels of abiotic background noise and calling in synchrony with other frogs in the chorus (Tuttle and Ryan 1982; Legett et al. 2019). Similarly, eavesdroppers may impose different selective pressure among different call types within a species (Bernal et al. 2006). Our results suggest that, given eavesdropper bias for Type I call, the cost of producing Type I calls may be greater than Type II calls for male Ryukyu Kajika frogs. However, to examine how eavesdropper selection may have shaped signaling behavior in Ryukyu Kajika frogs, the attractiveness of each call type to female Ryukyu Kajika frogs must also be considered. While previous studies suggest that both call types may attract mates (Tang 2009; Wang et al. 2017), their relative attraction and exact social function is unknown. Future studies examining tradeoffs in the attraction of mates and eavesdropping flies in this system might prove valuable in understanding the role that frog-biting mosquitoes and midges play in signal repertoire evolution.

Table 4.1. Numbers of insects collected by frog call traps broadcasting either Type I or Type II Ryukyu Kajika frog calls. Mosquitoes (Culicidae) and midges (Corethrellidae) were analyzed using GLMMs with zero-inflated Poisson error structures, moth flies (Psychodidae) were analyzed using a GLMM with a non-zero-inflated error structure, and non-eavesdropping insects (Bycatch) were analyzed using a GLMM with a negative binomial II error structure ($n = 30$ nights, $df = 53$). Statistically significant differences ($p < 0.05$) are indicated with a “*”.

Family	Species	Insects attracted to calls		z-value	p-value	effect size
		Type I	Type II			
Culicidae	<i>Ur. (Pseudoficalibia)</i>					
	<i>nivipleura</i>	1	0	-	-	-
	<i>ohamai</i>	6	5	-	-	-
	<i>Ur. (Ura.)</i>					
	<i>annandalei</i>	12	5	-	-	-
	<i>macfarlanei</i>	65	6	5.34	< 0.001*	2.56
	Total <i>Ur.</i>	84	16	4.25	< 0.001*	1.66
Corethrellidae	<i>Corethrella nippon</i>	60	14	2.80	0.005*	1.37
Psychodidae	<i>spp.</i>	22	15	1.14	0.253	0.38
Bycatch		177	257	-0.90	0.374	-0.19

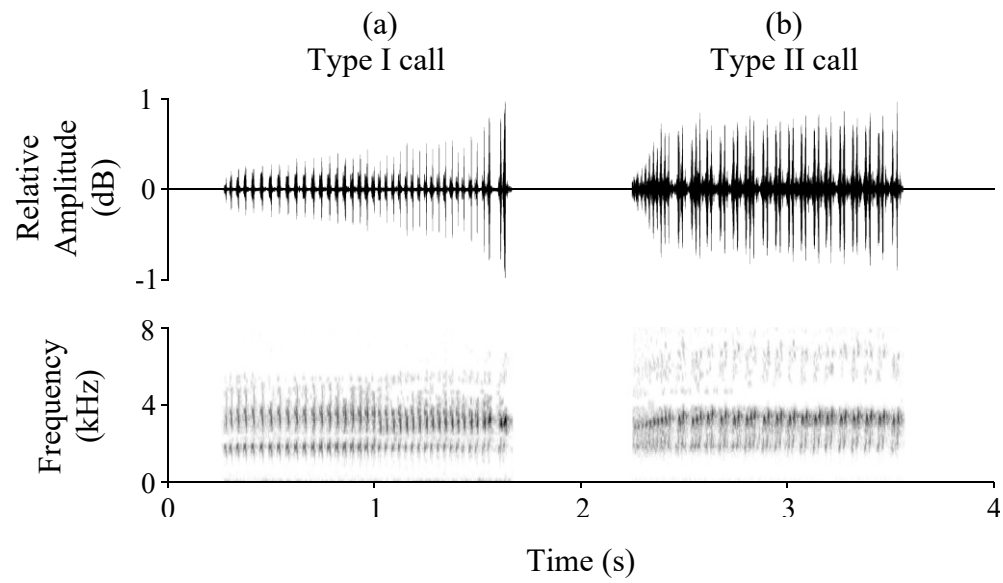


Figure 4.1. Oscillogram (top) and spectrogram (bottom) of a Type I (a) and Type II (b) Ryukyu Kajika frog call.

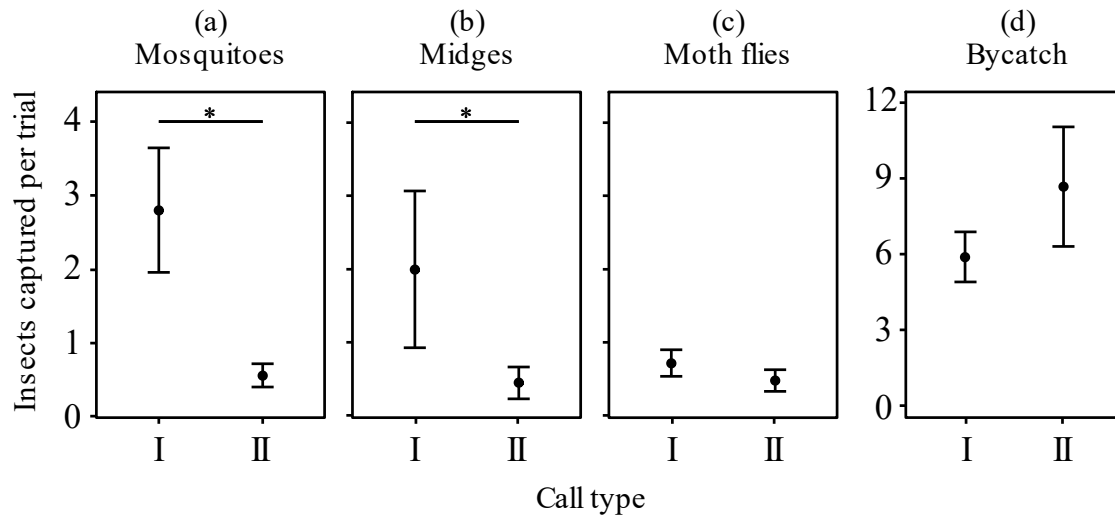


Figure 4.2. Frog-biting mosquitoes of the genus *Uranotaenia* (a), frog-biting midges of the genus *Corethrella* (b), moth flies (c), and bycatch (d) attracted to either Type I or Type II Ryukyu Kajika frog calls. Values are means and standard errors. Statistically significant differences between call types ($p < 0.05$) are indicated with a “*”. Note the difference in scale for bycatch.

4.6 References

- Albert, J. T., and Kozlov, A. S. (2016). Comparative aspects of hearing in vertebrates and insects with antennal ears. *Current Biology* 26: R1050-R1061.
- Bernal, X. E., and Pinto, C. M. (2016). Sexual differences in prevalence of a new species of trypanosome infecting túngara frogs. *International Journal for Parasitology: Parasites and Wildlife* 5: 40-47.
- Bernal, X. E., Rand, A. S., and Ryan, M. J. (2006). Acoustic preferences and localization performance of blood-sucking flies (*Corethrella* Coquillett) to túngara frog calls. *Behavioral Ecology* 17: 709-715.
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., and White, J. S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* 24: 127-135.
- Borkent, A. (2008). The frog-biting midges of the world (Corethrellidae: Diptera). *Zootaxa* 1804: 1-456.
- Borkent, A., and Belton, P. (2006). Attraction of female *Uranotaenia lowii* (Diptera: Culicidae) to frog calls in Costa Rica. *The Canadian Entomologist* 138: 91-94.
- Bravo, F., and Salazar-Valenzuela, D. (2009). A new species of *Sycorax* Curtis (Diptera, Psychodidae, Sycoracinae) collected on harlequin frogs (Anura: Bufonidae, Atelopus) in the Ecuadorian Andes. *Zootaxa* 2093: 37-42.
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., and Bolker, B. M. (2017). *glmmTMB* balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* 9: 378-400.
- Camp, J. V. (2006). *Host attraction and host selection in the family Corethrellidae (Wood and Borkent)(Diptera)*. Unpublished master's thesis. Georgia Southern University, Statesboro.
- Cutajar, T. P., and Rowley, J. J. (2020). Surveying frogs from the bellies of their parasites: Invertebrate-derived DNA as a novel survey method for frogs. *Global Ecology and Conservation* 22: e00978.
- de Silva P., Bernal, X.E., Chathuranga W.G. D, Herath, B.P., Ekanayake, C, Abeysundara, H.T.K., and Karunaratne, S.H.P.P. (in press). Feeding patterns revealed host partitioning in a community of frog-biting mosquitoes. *Ecological Entomology*.
- de Silva, P., Nutter, B., and Bernal, X. E. (2015). Use of acoustic signals in mating in an eavesdropping frog-biting midge. *Animal Behaviour* 103: 45-51.
- Downes, J. A. (1969). The swarming and mating flight of Diptera. *Annual review of entomology* 14: 271-298.
- Duellman, W. E., and Trueb, L. (1986). *Biology of amphibians*. McGraw-Hill Book Co., New York.
- Fox J, Weisberg S (2019). *An R Companion to Applied Regression*, Third edition. Sage, Thousand Oaks CA.
- Gopfert, M. C., and Robert, D. (2001). Active auditory mechanics in mosquitoes. *Proceedings of the Royal Society B* 268: 333-339.
- Gopfert, M. C., Briegel, H., and Robert, D. (1999). Mosquito hearing: sound-induced antennal vibrations in male and female *Aedes aegypti*. *Journal of Experimental Biology* 202: 2727-2738.
- Hartig, F. (2018). *DHARMa*: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package v. 0.2. 0.

- Johnson, R. N., Young, D. G., and Butler, J. F. (1993). Trypanosome transmission by *Corethrella wirthi* (Diptera: Chaoboridae) to the green treefrog, *Hyla cinerea* (Anura: Hylidae). *Journal of Medical Entomology* 30: 918-921.
- Kuramoto, M. (1986). Call structures of the rhacophorid frogs from Taiwan. *Scientific report of the Laboratory for Amphibian Biology, Hiroshima University* 8: 45-68.
- Legett, H. D., Baranov, V. A., and Bernal, X. E. (2018). Seasonal variation in abundance and diversity of eavesdropping frog-biting midges (Diptera, Corethrellidae) in a neotropical rainforest. *Ecological Entomology* 43: 226-233.
- Legett, H. D., Page, R. A., and Bernal, X. E. (2019). Synchronized mating signals in a communication network: the challenge of avoiding predators while attracting mates. *Proceedings of the Royal Society B* 286: 20191067.
- Lehane, M. J. (2005). *The biology of blood-sucking in insects*. Cambridge University Press.
- Mačát, Z., Sah, H. H. A., and Grafe, T. U. (2015). Preliminary results from observation of blood-sucking flies genus *Sycorax* (Diptera: Psychodidae) hosted by *Ansonia longidigita* (Anura: Bufonidae) in Borneo. Unpublished data.
- McKeever S. 1977. Observations of *Corethrella* feeding on tree frogs (*Hyla*). *Mosquito News* 37:522-523.
- McKeever, S., and Hartberg, W. K. (1980). An effective method for trapping adult female *Corethrella* (Diptera: Chaoboridae). *Mosquito News* 20: 111-112.
- Menda, G., Nitzany, E. I., Shamble, P. S., Wells, A., Harrington, L. C., Miles, R. N., and Hoy, R. R. (2019). The long and short of hearing in the mosquito *Aedes aegypti*. *Current Biology* 29: 709-714.
- Meuche, I., Keller, A., Ahmad Sah, H. H., Ahmad, N., and Grafe, T. U. (2016). Silent listeners: can preferences of eavesdropping midges predict their hosts' parasitism risk?. *Behavioral Ecology* 27: 995-1003.
- Miyagi, I., and Toma, T. (2013). *Uranotaenia* (Pseudoficalbia) *tanakai* (Diptera, Culicidae), a new species from forest swamps, Iriomote Is., the Ryukyu Archipelago, Japan. *Medical Entomology and Zoology* 64: 167-174.
- Nadrowski, B., Effertz, T., Senthilan, P. R., and Göpfert, M. C. (2011). Antennal hearing in insects—new findings, new questions. *Hearing research* 273: 7-13.
- Page, R. A., Ryan, M. J., and Bernal, X. E. (2014). Be loved, be prey, be eaten. In *Animal behavior case studies: integration and application of animal behavior, Vol. 3* (ed. K Yasukawa), pp. 123–154. Praeger, New York.
- Takken, W., and Verhulst, N. O. (2013). Host preferences of blood-feeding mosquitoes. *Annual Review of Entomology* 58: 433-453.
- Tang, W. (2009). *The chorus sound characteristics and phonotactic behavior of Buergeria japonica*. Unpublished master's thesis. National Cheng Kung University, Tainan City.
- Toma, T., Miyagi, I., Higa, Y., Okazawa, T., and Sasaki, H. (2005). Culicid and Chaoborid flies (Diptera: Culicidae and Chaoboridae) attracted to a CDC miniature frog call trap at Iriomote Island, the Ryukyu Archipelago, Japan. *Medical Entomology and Zoology* 56: 65-71.
- Toma, T., Takara, T., Miyagi, I., Futami, K., and Higa, Y. (2019). Mosquitoes and frog-biting midges (Diptera: Culicidae and Corethrellidae) attracted to traps with natural frog calls and synthesized sounds at Iriomote Island, Ryukyu Archipelago, Japan. *Medical Entomology and Zoology* 70: 221-234.

- Tuttle, M. D., and Ryan, M. J. (1982). The role of synchronized calling, ambient light, and ambient noise, in anti-bat-predator behavior of a treefrog. *Behavioral Ecology and Sociobiology* 11: 125-131.
- Virgo, J., Ruppert, A., Lampert, K. P., Grafe, T. U., and Eltz, T. (2019). The sound of a blood meal: Acoustic ecology of frog-biting midges (*Corethrella*) in lowland Pacific Costa Rica. *Ethology* 125: 465-475.
- Wang, Y. H., Hsiao, Y. W., Lee, K. H., Tseng, H. Y., Lin, Y. P., Komaki, S., and Lin, S. M. (2017). Acoustic differentiation and behavioral response reveals cryptic species within *Buergeria* treefrogs (Anura, Rhacophoridae) from Taiwan. *PloS one* 12: e0184005.

CHAPTER 5. THE DUAL BENEFITS OF SYNCHRONIZED MATING SIGNALS IN A JAPANESE TREEFROG: ATTRACTING MATES AND MANIPULATING PREDATORS

H. D. Legett¹, I. Aihara², X. E. Bernal^{1,3}

¹ *Purdue University, West Lafayette, IN, USA*

² *University of Tsukuba, Tsukuba, Ibaraki, JP*

³ *Smithsonian Tropical Research Institute, Balboa, Ancón, Panama, PA*

5.1 Abstract

In dense mating aggregations, such as leks and choruses, acoustic signals produced by competing male conspecifics often overlap. Overlapped signals reduce the ability of the females to discriminate between individual signals. Yet, despite this cost, males of some species deliberately overlap their signals, synchronizing signal production in the chorus. Here, we investigate two non-mutually exclusive hypotheses of synchronized mating signals in a Japanese stream breeding treefrog (*Buergeria japonica*): 1) increased female attraction to the chorus through acoustic constructive interference (the beacon effect hypothesis) and 2) reduced attraction of eavesdropping predators through perceptual manipulation (the eavesdropper avoidance hypothesis). Our results from field playback experiments on female frogs and eavesdropping predators (midges and mosquitoes) support both hypotheses. Signal transmission and female phonotaxis experiments suggest that synchronized calls are more attractive to females at a distance than unsynchronized calls. Eavesdropper and additional female phonotaxis experiments suggest that synchronized calls reduce eavesdropper attraction through perceptual manipulation while female attraction to individual signals is not affected. Therefore, synchronized signaling likely benefits male *B. japonica* in multiple ways. These findings highlight the complex nature of signal synchronization and how multiple interacting selective pressures likely have promoted that the evolution and maintenance of this behavior.

5.2 Introduction

Males of many species aggregate and produce conspicuous displays to attract mates. Examples of this phenomenon occur across taxa in the choruses and leks of mammals, birds, insects, and anurans. Attracting mates in aggregations can be challenging, however, as mating signals produced at the same time among neighboring males can overlap and acoustically interfere, reducing the ability of females to detect, localize, and discriminate between signals (Wollerman 1999; Wollerman and Wiley 2002a; Wollerman and Wiley 2002b). In addition, when acoustic signals overlap but are offset, females often exhibit a bias for the initial ‘leading’ signal over the second ‘following’ signal. Known as the ‘precedence effect’ (Wallach et al. 1949), or leader-follower preferences, this bias can override other acoustic preferences in female receivers (Whitney and Krebs 1975; Klump and Gerhardt 1992; Grafe 1996; Greenfield et al. 1997; Bosch and Márquez 2002; Höbel 2010). Given the costs of signal overlap, evolution by sexual selection is expected to result in signal timing strategies that reduce signal overlap. In anurans choruses, for example, conspecific neighboring males commonly offset the timing of signal production in an alternating pattern, forming duets, trios, and quartets (Duellman 1970). Similar patterns of signal overlap avoidance are common in insects (Gerhardt and Huber 2002) and birds (Farabaugh 1982) and in mating signals produced in other modalities (Carlson and Copeland 1985; Morin 1986).

While alternation of signals is widespread, there is considerable variation in signal timing strategies across species (Wells and Schwartz 2007). Opposite to signal alternation, males of some species produce calls in ‘synchrony’, deliberately overlapping their mating signals with neighboring conspecifics in a chorus (insects and anurans: Greenfield 1994; birds: Hall 2009; mammals: Harrington and Mech 1979; crustaceans: Reaney et al. 2008). Furthermore, in many synchronizing anuran and insect species, males actively produce following signals in response to the signals of neighboring males (Ryan 1986; Grafe 1999; Hartbauer et al. 2005). Unlike the benefits of mating signal alternation, increased female attraction to the individual, the benefits of synchrony are less understood. Furthermore, any advantages males might gain from synchronizing would have to weigh against the cost of reduced female attraction, both from signal interference and leader-follower preferences.

Several hypotheses have been proposed to explain the use of synchronized mating signals. When signals overlap, constructive interference may increase the peak amplitude of the combined signals, creating an acoustic ‘beacon’ (Buck and Buck 1978; Greenfield 1994). The beacon effect function of signal synchrony could increase female attraction in multiple related ways. In general, females may prefer signals of higher intensity (Ryan and Keddy-Hector 1992). Overlapping signals with increased amplitude could also have a greater active space, resulting in the attraction of females from a greater distance (Gerhardt 1975; Penna and Solis 1998; Gerhardt and Klump 1988). The beacon effect is only advantageous, however, if the *per capita* increase in female attraction is higher for aggregations producing synchronized signals compared to unsynchronized ones (Greenfield 1994). An equivalent tradeoff has received some attention in relation to group size and the evolution of aggregate breeding (Ryan et al. 1981; Kokko et al. 1998). Larger aggregations of male signalers attract more females, but chorus size is limited by the increase in male-male competition and *per capita* mating advantage (Greenfield 1994). In this case, the selective forces that drive group size dynamics and signal timing strategies are analogous.

Synchronized mating signals may also benefit males in evading eavesdropping predators and parasites (Tuttle and Ryan 1982). The conspicuousness of mating displays often exposes signalers to nontarget receivers, including natural enemies (Zuk and Kolluru 1998; McGregor and Peake 2000). Just as signal overlap may reduce female attraction through signal interference and leader-follower preferences, so too might it reduce the attraction of eavesdropping predators and parasites. Indeed, previous studies on synchronizing frogs found that predators prefer unsynchronized frog calls over synchronized calls (Tuttle and Ryan 1982; Legett et al. 2019), and bias leading over following calls (Legett et al. 2020). Thus, by producing following overlapped signals in response to calls produced by neighboring conspecifics, males may mask their own signals and exploit the perceptual biases of their enemies.

Here, we investigate these two non-mutually exclusive functions of mating signal synchronization, the beacon effect and eavesdropper avoidance hypotheses. Specifically, we conduct a series of field playback experiments to examine the benefits of synchronized calling in a Japanese treefrog, the Ryukyu Kajika frog (*Buergeria japonica*). To test the beacon effect

hypothesis, we compare how synchronized and unsynchronized calls transmit through the environment. We also assess female preference for synchronized and unsynchronized calls at a distance from the chorus. To test the eavesdropper avoidance hypothesis, we assess the leader-follower preferences of predators that eavesdrop on *B. japonica* calls. Finally, we weigh these potential benefits of signal synchrony against one potential cost: reduced female attraction through the leader-follower preferences of female *B. japonica*.

5.3 Methods

5.3.1 Study system and location

The Ryukyu Kajika frog (*B. japonica*), in the Rhacophoridae family, ranges from Taiwan through the Ryukyu Archipelago of Japan. During their breeding season, March to October, males of this species form choruses along the banks of streams and roadside ditches (Chen et al. 2001). Chorus attendance is correlated with rainfall, and large choruses of over 300 males have been observed on night following periods of heavy rains (Legett et al. 2020). Although specific calling behavior is variable between populations, calls are generally divided into two types, one of which is produced in synchrony (Legett et al. 2020). This synchronized call is a trill of variable duration (from < 0.5 s to > 3.0 s: Kuramoto 1986) with a dominant frequency around 3 kHz and likely plays a role in attracting mates (Tang 2009; Wang et al. 2017). Male *B. japonica* respond to the calls of neighboring males at an average latency of 0.35 ± 0.05 s, resulting in around 70 % overlap in call duration between leading and following calls (Legett et al. 2020). In addition, *B. japonica* calls attract multiple species of eavesdropping insects (Figure 5.1a), frog-biting midges (*Corethrella* spp.) and mosquitoes (*Uranotaenia* spp.), which take blood meals from calling males (Toma et al. 2014; Toma et al. 2019). The costs of attracting frog-biting insects include blood loss (Camp 2006) and spread of diseases (Johnson et al. 1993; Bernal and Pinto 2016; Camp et al. 2018). All field experiments were conducted in July and August 2016 on Iriomote Island in southern Okinawa, Japan ($24^{\circ}23'30.3''\text{N}$, $123^{\circ}52'48.8''\text{E}$).

5.3.2 Acoustic playbacks

For each experiment, acoustic playbacks of synthetic *B. japonica* calls were broadcast using Pignose portable amplifier speakers (Model 7-100) at a peak amplitude of 80 dB SPL re. 20 μP

(Brüel and Kjær digital sound level meter Type 2250) measured at 1 m from the speaker at ground level. Speakers were paired in sets, spaced 1 m apart, to broadcast either synchronized (0.30 s of latency) or unsynchronized (alternating) calls at a rate of one call every 3.4 s. The synthetic *B. japonica* call was constructed in CoolEdit2000 (Syntrillium Software) using a representative note from a pre-recorded *B. japonica* call. The synthetic call was designed to roughly match the average characteristics of male calls in this population (dominant frequency = 3.25 kHz, call duration = 1.62 s; Table 5.1, Figure 5.1b).

5.3.3 Signal transmission

As acoustic signals transmit through the environment they attenuate (reduce in amplitude) and degrade in their spectro-temporal characteristics. We compared these aspects of signal transmission by broadcasting synchronized and unsynchronized *B. japonica* calls then rerecording these broadcasts at a distance of 1, 2, 3, 4, 5, 10, 20, 30, 40, 60, and 80 m from the speakers (following previously established methods, Kime et al. 2000). This signal transmission experiment was conducted at five interior forest sites. Each site was tested once ($n = 5$). Fast Fourier transformation (FFT) recordings of the transmitted calls were collected using a Brüel and Kjær digital sound level meter Type 2250 (10 s recording, 50 Hz frequency resolution over a range of 500 – 6000 Hz). Differences in synchronized and unsynchronized call attenuation were assessed by comparing the peak amplitude at the dominant frequency (3.25 kHz) at each distance. Differences in spectral degradation were assessed by comparing cross-correlation coefficients of calls at each distance. Coefficients were calculated using a Pearson correlation analysis comparing the FFT recording at a given distance to the recording taken at 1 m.

5.3.4 Female synchronized-unsynchronized call preference

Following standard procedures used in phonotaxis experiments with female frogs (e.g. Ryan and Rand 1990), pairs of male and female *B. japonica* were collected in amplexus from a naturally occurring chorus. Just prior to testing, females ($n = 24$) were separated from the male and positioned under a cup at the center of a circular phonotaxis arena. The arena was 2 m in diameter with open sides marked at 15° increments, located in a haphazardly selected forest site at a distance greater than 100 m from any breeding *B. japonica*. Two sets of speakers were

placed on either side of the arena (180° from each other) at a distance 40 m from the arena's center. One speaker set broadcast synchronized calls while the other broadcast unsynchronized calls. While the speaker sets were always placed at 180° from each other, the position of the sets relative to the arena were repositioned between trials to control for side biases. The arena was illuminated using red light, and female movement in the arena was observed from a blind placed perpendicular to the speakers. Females were given 5 min to acclimate to the arena under the cup, before being remotely released. The point at which the female left the arena to the closest 15° mark was recorded. Females were tested once and then returned with their paired male to their exact collection location.

5.3.5 Leading-following call preferences in eavesdropping micropredators

Frog-biting midges and mosquitoes were collected using standard sound traps for insects (McKeever and Hartberg 1980). A collection device was placed over each speaker in a pair spaced 1 m apart broadcasting synchronized calls. Within a trial, one speaker always broadcast leading calls while the other always broadcast following calls. Which speaker broadcast which stimuli, leading or following calls, was randomized between trials. Collection sites were haphazardly chosen in the forest within 100 m of a water source. Traps were run once per night for 45 min starting 30 min after sunset (Legett et al. 2019). Over a sampling period of 20 nights, a total of 97 frog-biting midges and 37 frog-biting mosquitoes were collected. At the end of each night, frog-biting insects were counted and preserved in 75% ethanol. Species were identified using established keys (*Corethrella*: Borkent 2008; *Uranotaenia*: Miyagi and Toma 2013). The number of mosquitoes and midges attracted each night to either the leading or following calls was used as an indicator of preference.

5.3.6 Leading-following call preferences in female frogs

Following the previously described procedures for phonotaxis experiments with female frogs, female *B. japonica* leading-following call preferences were assessed in a 2 m diameter open sided circular phonotaxis arena. Briefly, females were captured ($n = 14$), placed under a cup at the center of the arena and given 5 min to acclimate before being released remotely. A pair of speakers spaced 1 m apart broadcasting synchronized calls was placed at a distance 2 m from the

center of the arena. Which speaker broadcast leading or following calls was randomized between trials. Female choice was scored after the female approached within 10 cm of a speaker (Ryan and Rand 1990). Females were tested once and then returned to their exact collection locations with their paired males.

5.3.7 Statistical analyses

All statistical analyses were conducted using program R version 3.5.2 (R Development Core Team 2015). To assess differences in call transmission for synchronized and unsynchronized calls, changes in peak amplitude and Pearson correlation coefficients were compared using Linear Mixed Models (LMMs) in the *glmmTMB* package (Brooks et al. 2017). The interaction between distance (1, 2, 3, 4, 5, 10, 20, 30, 40, 60, and 80 m) and call overlap (synchronized or unsynchronized) was included as a fixed factor and site was included as a random factor. Differences in the slopes of the covariates in each model, the change in values over distance, were compared using the *emtrends* function in the *emmeans* package (Lenth et al. 2018). Pearson correlation coefficients of calls at each distance were calculated using the standard *cor* function in R. To assess female *B. japonica* preference for synchronized-unsynchronized calls, the circular direction that females left the arena (to the nearest 15° increment) were analyzed using the *CircStats* package (Lund and Agostinelli 2018). Parameters for the von Mises distribution of the angles, mean (μ) and dispersion (κ), were calculated from maximum likelihood estimates. A Rayleigh test of uniformity was used to test the null hypothesis that females left the arena at a random distribution (Humphreys and Ruxton 2017). Leading-following call preferences in eavesdropping predators were analyzed using two-tailed exact symmetry tests in the *coin* R package (Hothorn et al. 2006), testing the null hypothesis that leading and following calls attracted equal numbers of frog-biting insects (following Legett et al. 2020). The number of captured frog-biting mosquitoes and frog-biting midges were independently compared, blocked within each night. Finally, the leading-following call preferences of female *B. japonica* were assessed using a two-tailed exact binomial test. Given the small sample size for the female leading-following preference experiment, concerns about type II error were assessed using a G*Power 3.1 statistical power (Faul et al. 2009).

5.4 Results

5.4.1 Signal transmission

The differences in peak amplitude between synchronized and unsynchronized *B. japonica* calls was variable over distance. On average, however, the dominant frequency of synchronized calls was more intense by 2.14 dB (Table 5.2a). Calls attenuated at a rate relative to the square root of distance, with no significant difference between synchronized and unsynchronized calls (attenuation rate synchronized = -7.18 ± 0.27 , attenuation rate unsynchronized = -7.05 ± 0.27 ; $t(100) = -0.35$, $p = 0.729$; Figure 5.2a). Calls spectrally degraded at a linear rate, also with no significant difference between synchronized and unsynchronized calls (both rates = $-0.01 \pm < 0.01$, $t(100) = 1.79$, $p = 0.076$; Table 5.2b, Figure 5.2b).

5.4.2 Female synchronized-unsynchronized call preference

Female *B. japonica* displayed a bias for sets of synchronized calls over unsynchronized calls broadcast at a 40 m distance. When the synchronized speakers are positioned at 0° , and the unsynchronized speakers positioned at 180° , females exited the phonotaxis arena at a mean angle of 4.96° ($\kappa = 1.13$), significantly oriented towards the synchronized speaker set (mean resultant length = 0.484, $p < 0.001$; Figure 5.3).

5.4.3 Leading-following call preferences in eavesdropping micropredators

Overall, the number of eavesdropping insects collected per night was low, < 10 individuals on average. Leading speakers attracted about 2 more frog-biting midges per night compared to speakers broadcasting following calls (3.30 ± 2.62 midges for leading calls vs. 1.35 ± 1.66 midges for following calls; $Z = 2.65$, $p = 0.004$; Figure 5.4a). No significant difference in the attraction of frog-biting mosquitoes was detected between speakers (1.15 ± 1.14 mosquitoes for leading calls vs. 0.90 ± 0.64 mosquitoes for following calls; $Z = 0.85$, $p = 0.514$; Figure 5.4b).

5.4.4 Leading-following call preferences in female frogs

Female *B. japonica* displayed no significant preference for leading or following calls, with only 6 of the 14 females tested choosing the leading speaker ($p = 0.791$; Figure 5.5). This result

contrasts with previous experiments examining leading-following call preferences in other frog species have found strong leading call preferences (Schwartz 1986, Dyson and Passmore 1988, Grafe 1996, Bosch and Márquez 2002, Marshall and Gerhardt 2010, Reichert et al. 2016, Legett et al. 2019). Using the average effect size from these studies, our female phonotaxis experiment had statistical power $1 - \beta = 0.69$ ($\alpha = 0.05$, effect size = 0.360). If female *B. japonica* had leading-following call preferences comparable to other anuran species, we might expect 12 of the 14 females tested to choose leading calls.

5.5 Discussion

Our results support both functions of synchronized mating signals, the beacon effect and as a predator avoidance strategy. Although we found few acoustic differences in the transmission of synchronized and unsynchronized frog calls across the forest, female *B. japonica* displayed a bias for synchronized calls at a distance beyond the chorus. These findings suggest that a group of synchronizing male *B. japonica* may enjoy an advantage over a group of unsynchronized males in attracting female frogs. In addition, our results show that individuals within a synchronized chorus that produce following calls attract fewer frog-biting midges compared to individuals producing leading calls. Thus, eavesdroppers of *B. japonica* calls display a precedence effect, suggesting that male *B. japonica* may reduce the attraction of at least some micropredators to their individual calls by synchronizing. Furthermore, by also assessing leading-following call preferences in female *B. japonica*, we weigh both advantages of synchrony against the cost of reduced female attraction. Female *B. japonica*, however, did not display a strong preference for leading calls. This result contrasts with the behavior of other anuran species (e.g. Marshall and Gerhardt 2010; Reichert et al. 2016), suggesting that female *B. japonica* have reduced leading-following call preferences, releasing males from this tradeoff of mating signal synchrony (Legett et al. 2019).

5.5.1 Signal synchronization as a ‘beacon’ for females

We found no evidence that synchronized and unsynchronized signals attenuate or degrade differently as they are transmitted through the environment. Synchronized *B. japonica* calls did constructively interfere, however, and were generally higher in amplitude compared to

unsynchronized calls at a given distance. As predicted, we found that females displayed a bias for pairs of synchronized calls over unsynchronized calls away from the chorus. It is presently unclear, however, why female *B. japonica* display this bias for synchronized calls. The bias could result from a preference for higher amplitude signals, a common phenomenon in other frog species (Ryan 1985; Gerhardt 1987) and in general for female receivers of mating signals across taxa (Ryan and Keddy-Hector 1992). In addition, synchronized signals can also result in a greater active space and are thus expected to increase the overall number of receivers attracted to the chorus. Given that sound radiation of frog calls is not omnidirectional and can be biased in directivity towards particular receivers (Bernal et al. 2009), further studies that examine radiation patterns in synchronizing species could provide valuable insights.

Previously, the beacon effect hypothesis has been used to explain the evolution of chorusing in general (Ryan et al. 1981). When animal aggregations are dense enough, signals unintentionally overlap and constructively interfere to create high amplitude ‘chorus noise’ which can be used by females to locate the chorus (Bee 2007). Intentional overlap of signals, therefore, may be a behavioral extension of this benefit. By synchronizing their calls, males further increase chorus noise, producing an even more intense beacon.

One aspect that can restrict the beacon effect function of signal synchronization is the prevalence of cheaters. A male producing unsynchronized calls within a synchronized chorus would benefit from increased female attraction to the chorus, while also being more attractive once females arrive to the chorus. If males would respond to cheaters by producing following synchronized calls, the calls of cheaters would still be more attractive assuming female frogs prefer leading calls. Thus, even if a synchronized chorusing strategy increases the *per capita* rate of female attraction, males producing following synchronized calls are at a disadvantage to cheaters and leading callers. There may, however, be habitats that limit the advantages of leaders and cheaters. In habitats with high background noise, such as those near running water, male calls may already be acoustically masked (Brumm and Slabbekoorn 2005). High levels of background noise reduce the ability of receivers to detect signals and select between preferred and nonpreferred signals (discussed in Endler 1992; Brumm and Slabbekoorn 2005). Through the beacon effect, synchronized calls may be more detectable in background noise compared to

unsynchronized calls (Marshall and Gerhardt unpublished data discussed in Gerhardt and Huber 2002). We, therefore, might expect to observe synchrony in species that chorus in such noisy environments. Indeed, synchronization has been observed in several frog species that call near waterfalls and streams, including *B. japonica*, pug-nosed tree frogs (*Smilisca sila*: Tuttle and Ryan 1982), and canyon tree frogs (*Hyla arenicolor*: Marshall and Gerhardt unpublished data discussed in Gerhardt and Huber 2002). Furthermore, if signal synchronization is an adaptation to noisy environments, synchronizing species may also be more resilient to other types of background noise, such as anthropogenic noise from aircraft and automobile traffic, which can negatively impact anuran behavior (Bee and Swanson 2007).

5.5.2 Synchronization as a predator avoidance strategy

Synchronized signals may reduce the attraction of eavesdropping predators if overlapping signals are harder to detect, recognize, and localize, and if the eavesdroppers prefer leading to following calls (Legett et al. 2019; Legett et al. in press). We found that frog-biting midges are biased towards leading calls. Thus, by responding to the calls of neighboring males with following calls, male *B. japonica* can exploit the leader-follower preferences of their eavesdroppers and reduce the risk of predation. Similar leading-following call preferences have been observed in eavesdropping predators in other systems, both insect and mammalian (Lee et al. 2009; Legett et al. 2020).

If *B. japonica* synchronize their calls to avoid eavesdroppers, we might expect high rates of eavesdropper attraction and thus high selective pressure from predators. However, only about 3 or 4 midges were captured per night over the 45 min collection period. In other systems, such as the calling frogs in the Neotropics, the same collection methods result in the capture of hundreds of frog-biting insects in the same 45 min period (Bernal et al. 2006; Legett et al. 2019). In addition, *B. japonica* produce two calls types, a synchronized call type (the focus of our study) and a call type that is produced in alternation between neighboring males (Legett et al. 2020). When both call types are broadcast without overlap, eavesdropping midges and mosquitoes prefer the alternating call type (Legett et al. unpublished data, see Chapter 4). If synchrony functions to avoid eavesdroppers, however, why would male *B. japonica* synchronize the call type that is less attractive to those eavesdroppers? Given the small benefit that males may gain

from avoiding eavesdropping predators in this system, it is unlikely that eavesdropper attraction is the primary selective pressure driving the use of synchronization in *B. japonica*. While the current abundance of eavesdroppers may not represent past selective pressures, our results suggest a greater payoff from the beacon effect function of synchrony, while avoiding eavesdroppers may be a tangential benefit.

5.5.3 Relaxed sexual selection as a driver of signal synchronization

Synchronization of mating signals may provide multiple benefits to male *B. japonica*, but at the assumed cost of reduced short-distance female attraction to individual signals. It is possible, however, that relaxed female selection for unsynchronized signals could release males from this tradeoff imposed on signal synchrony (Legett et al. 2019). Our results indicate that female *B. japonica* do not display strong leading-following call preferences, suggesting that in this species males may enjoy the benefits of producing following synchronized calls without incurring this cost. The precedence effect is a widespread phenomenon among animals that acoustically communicate (e.g. Wytenbach and Hoy 1993; Dent and Dooling 2004; Marshall and Gerhardt 2010; Brown et al. 2015). It is intriguing that reduced or reversed leader-follower preferences have been observed in other synchronizing anuran species in addition to *B. japonica* (*Dendropsophus ebraccatus*: Wells and Schwartz 1984; *Kassina fusca*: Grafe 1999; *Smilisca sila*: Legett et al. 2020). Thus, while the potential benefit of mating signal synchronization may be enjoyed by both synchronous and nonsynchronous species (Legett et al. 2019), it is relaxed sexual selection that provides signaling males the opportunity to take advantage of synchrony.

The interpretations of our results for the female leading-following preference experiment are limited by our small sample size. While these results are suggestive, a more robust female choice experiment with varying degrees of overlap between signals is needed to better understand female signal preferences in this species. In addition, auditory physiology experiments comparing synchronous and nonsynchronous species, specifically differences in temporal processing, are needed to understand exactly how females of some species overcome the precedence effect.

5.5.4 Signal synchronization in anurans compared to other tax

Synchronized signaling is assumed to be relatively uncommon in anurans, having only been reported in a handful of species in addition to *B. japonica* (*S. sila*: Ryan 1986; *Kassina senegalensis*: Wickler and Seibt 1974; *D. ebraccatus*: Wells and Schwartz 1984; *Cochranella granulose*: Ibáñez 1993; *K. fusca*: Grafe 1999; *Kassina kuvangensis*: Grafe 2003; *Hyla arenicolor*: Marshall and Gerhardt unpublished data; *Assa darlingtoni*: Clulow et al. 2017; *Diasporus diastema*: Capshaw et al. 2020). However, this rarity may be due to a bias in the studies describing natural history and signal timing strategies. For anurans, timing of signal production is often not reported with other standard call features, such as signal duration and dominant frequency. The signaling behavior of *B. japonica*, for example, has been the focus of previous studies (Kuramoto 1986; Wang et al. 2017), including the population on Iriomote Island (Tang 2009) where this study was performed. Yet, synchronization in *B. japonica* has only recently been described and discussed (Legett et al. 2020). Even in long-studied and common species, signal synchronization has only received limited consideration. The beacon effect function of signal synchrony in anurans was originally proposed for American toads (*Anaxyrus americanus*), which produce long trill calls that sometimes overlap (Wells 1977). Female preferences can be influenced by call overlap in this species (Howard and Palmer 1995), yet follow-up empirical studies examining when and why male *A. americanus* synchronize have not been conducted.

In contrast to anurans, synchronization of mating signals appears to be a more common strategy in insects, particularly crickets and katydids (Walker 1969; Shaw et al. 1990; Sismondo 1990; Greenfield and Roizen 1993; Nityanand and Balakrishnan 2007; Greenfield and Schul 2008; Schul et al. 2014). In large multi-species insect choruses, the beacon effect may benefit males by increasing the active space of signals and overcoming biotic background noise from the signals of other species (Shelly and Greenfield 1991; Greenfield 1994). In addition to increasing signal amplitude, synchronization may also benefit males by maintaining a species-specific rhythm, allowing females to more easily identify conspecific mates using this chorus level temporal cue (Greenfield 1994; Moiseff and Copeland 2010). Thus, for insects in this multi-species chorus context, the tradeoff driving signal synchronization is between interspecific competition for acoustic space and intraspecific competition.

Signal synchronization also occurs in groups other than anurans and insects. While these signals are often used in contexts other than mate attraction, synchronization often functions in similar ways. Packs of wolves (*Canis lupus*) and coyotes (*Canis latrans*), for example, may synchronize their howls to take advantage of the beacon effect (known in this context as the Beau Geste effect, McCarley 1975; Harrington 1989). Synchronized howls, which play a role in territorial maintenance, may make the size of the pack appear larger to distant receivers (Harrington 1989). Synchrony is used to avoid eavesdropping predators in other groups as well. Beaked whales (*Ziphius cavirostris*), for example, synchronize their echolocation signals to potentially reduce detection by killer whales (de Soto et al. 2020).

Finally, synchronized signaling is not limited to acoustic displays. Some fireflies (Buck and Buck 1966) and marine ostracods (Morin 1986) produce synchronized luminescent displays, preserving a species-specific rhythm and increasing group detectability through the beacon effect (Buck and Buck 1966; Buck and Buck 1978). Some species of fiddler crabs (*Uca* spp.) synchronize the courtship waving of their claws (Blackwell et al. 1998, Blackwell et al. 2006). Curiously, female fiddler crabs display a preference for leading claw waves over following call waves (Reaney et al. 2008), similar to the precedence effect in acoustic signals. Whether these visual displays help mask individuals from eavesdropping predators, however, has yet to be investigated. Overall, the advantages of signal synchrony, through the beacon effect and predator avoidance, are likely extended across signaling organisms and sensory modalities.

Table 5.1. Natural *B. japonica* call characteristics (dominant frequency and call duration) from previous studies compared to the characteristics of the synthetic call used in this study.

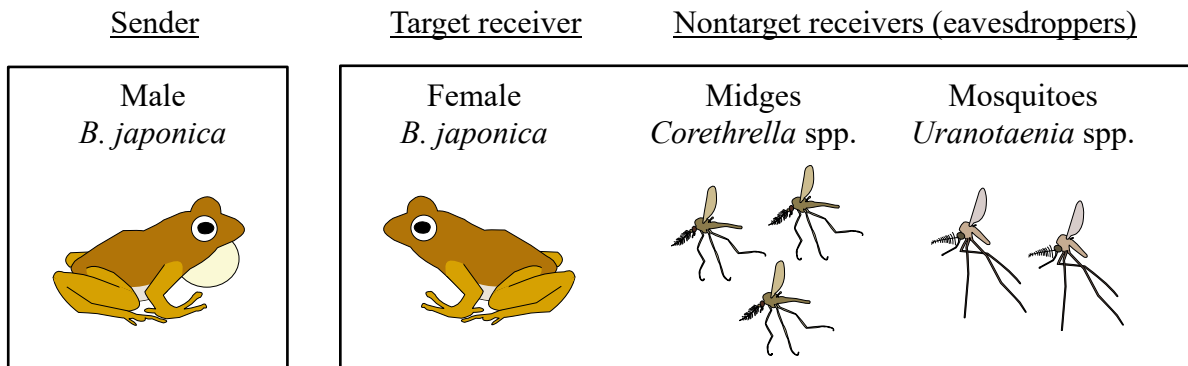
Natural <i>B. japonica</i> calls				Synthetic call
Location	Kuishanli, Taiwan	Iriomote Island, Japan	Iriomote Island, Japan	-
Sample size (n)	8	185	38	-
Dominant frequency (kHz)	3.13 ± 0.09	3.04 ± 0.15	3.34 ± 0.02	3.25
Call duration (s)	2.14 ± 0.21	0.68 ± 0.23	1.45 ± 0.08	1.63
Reference	Kuramoto 1986	Tang 2009	Legett et al. 2020	-

Table 5.2. Differences in signal attenuation (a) and spectral degradation (b) between broadcast synchronized and unsynchronized *B. japonica* calls rerecorded at distances of 1 – 80 m. Values are the average across five inner forest sites (mean $\pm$ se). Attenuation compares the amplitude (dB) of the dominant frequency of the calls, while degradation compares Pearson correlation coefficients.

(a)		Signal attenuation										
Distance (m)		1	2	3	4	5	10	20	30	40	60	80
synchronized – unsynchronized (dB)		1.77	5.22	-0.08	1.72	3.61	0.40	3.40	3.20	2.88	1.33	0.08
		±	±	±	±	±	±	±	±	±	±	±
		1.32	0.85	0.65	0.87	0.98	0.79	0.66	1.42	1.70	1.41	0.75

(b)		Signal degradation										
Distance (m)		1	2	3	4	5	10	20	30	40	60	80
synchronized – unsynchronized (corr. coeffs.)	0		-0.03	-0.03	-0.07	-0.03	-0.03	0.11	0.04	0.11	0.15	0.07
			±	±	±	±	±	±	±	±	±	±
			0.04	0.04	0.05	0.02	0.07	0.05	0.07	0.09	0.11	0.10

(a) Study system



(b) Mating signal

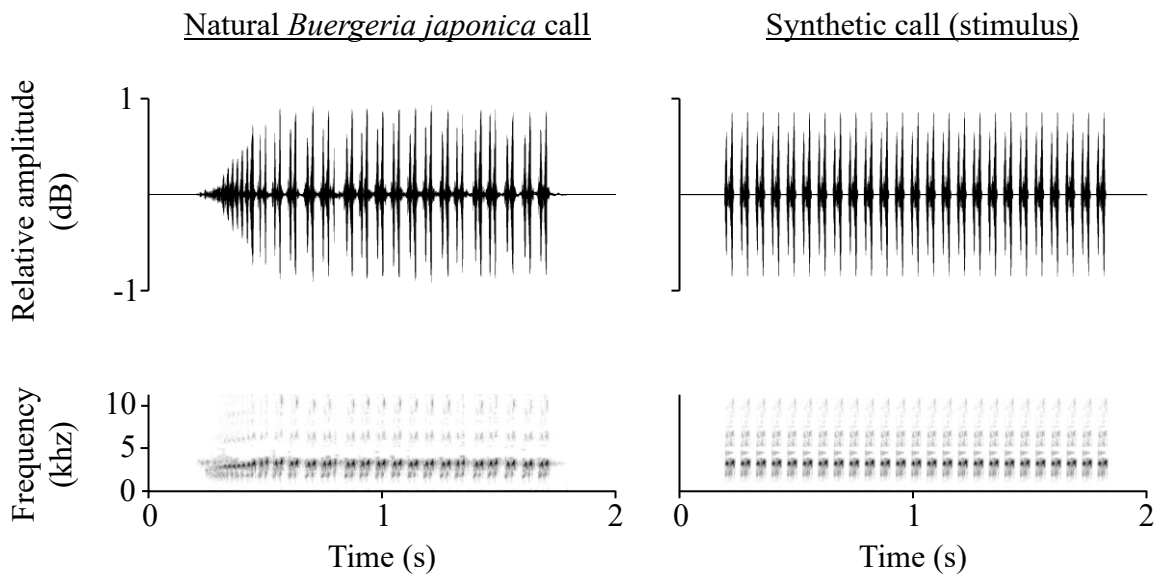


Figure 5.1. The study system (a), including the sender, target receiver, and nontarget receivers of *B. japonica* calls. Oscillograms and spectrograms (b) of a natural *B. japonica* call and the synthetic call used in this study.

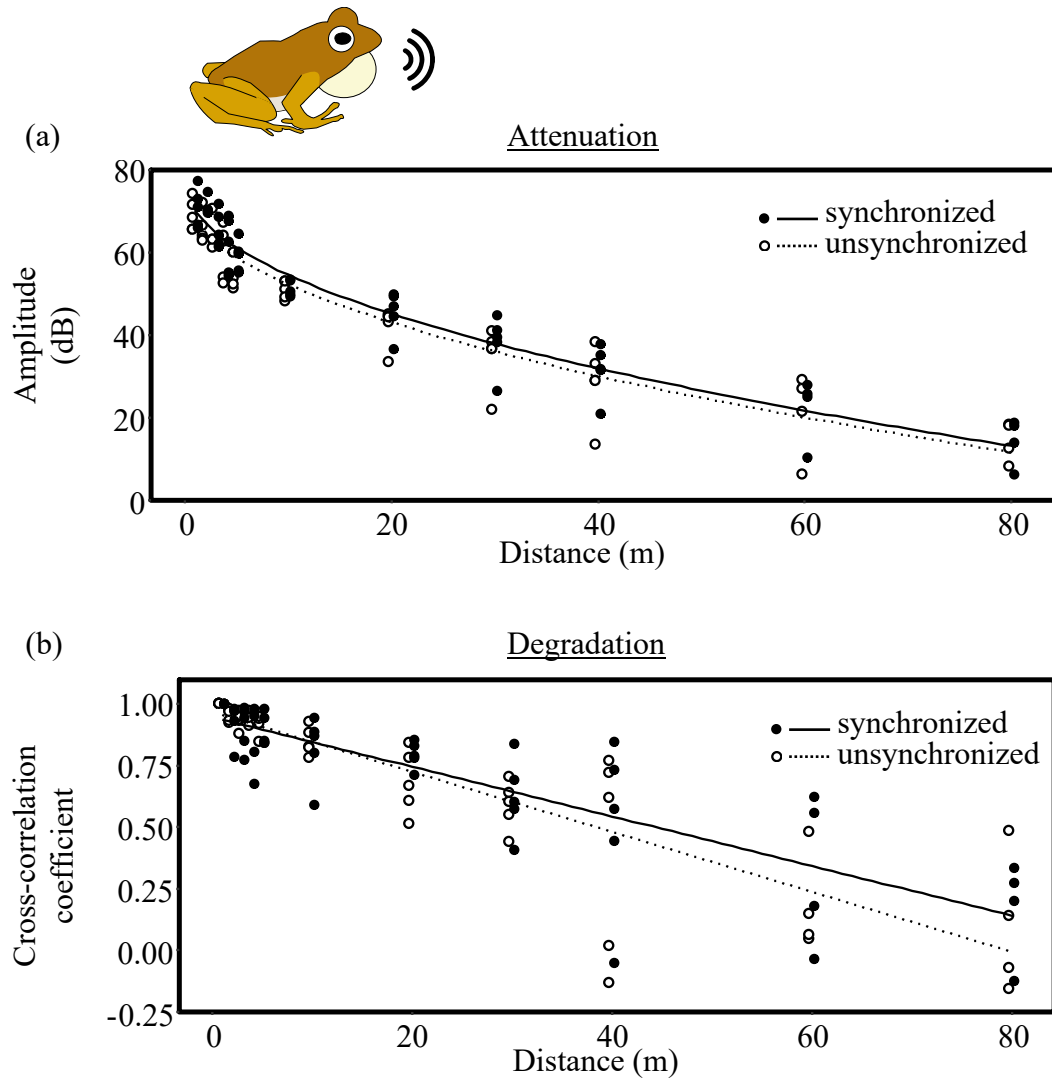


Figure 5.2. Attenuation (a) and degradation (b) of broadcast synchronized and unsynchronized *B. japonica* calls. Values for attenuation are the amplitude (dB) of the dominant frequency (3.25 kHz), while values for degradation are Pearson correlation coefficients.

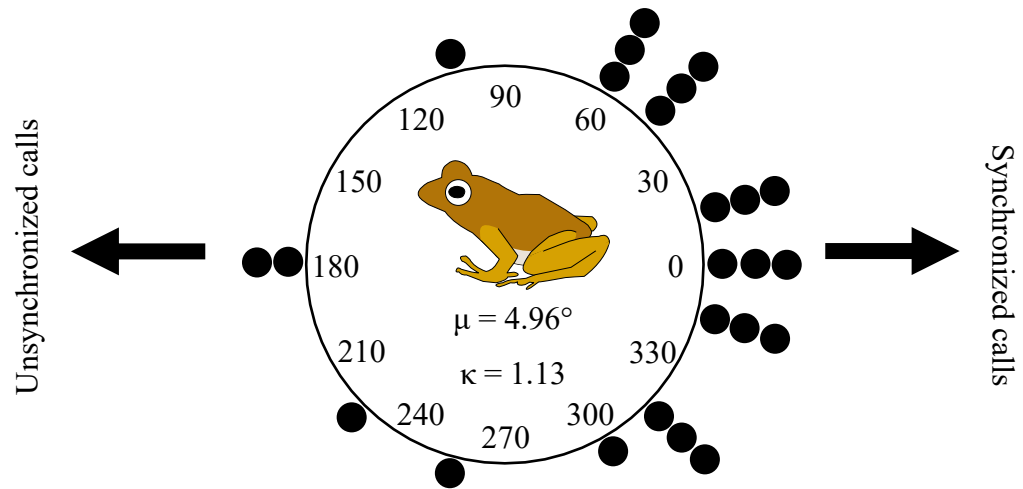


Figure 5.3. Circular diagram depicting the angle (to the nearest 15° increment) at which female *B. japonica* left the phonotaxis arena with synchronized calls broadcast at 0° and unsynchronized calls broadcast at 180°. Both stimuli were located at a distance 40 m from the center of the arena. Each circle represents an individual female frog.

Non-target receivers (eavesdroppers)

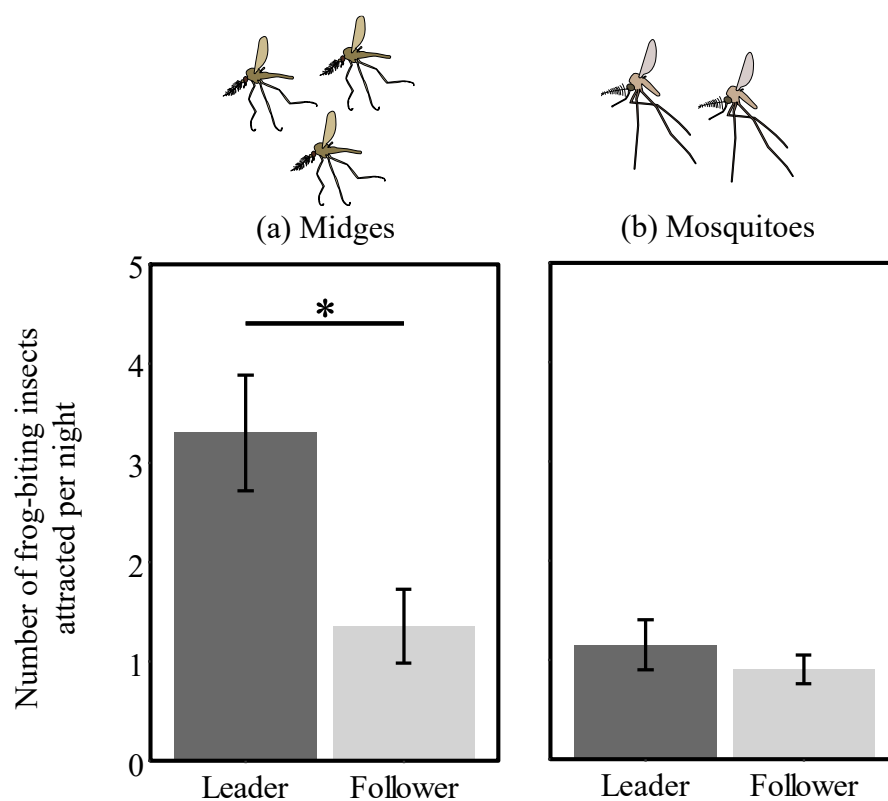
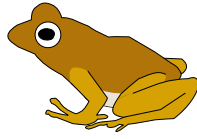


Figure 5.4. Eavesdropper preferences for leading or following calls. Numbers of midges (a) and mosquitoes (b) are the average collected per night (mean $\pm$ se).

Target receiver



Female
B. japonica

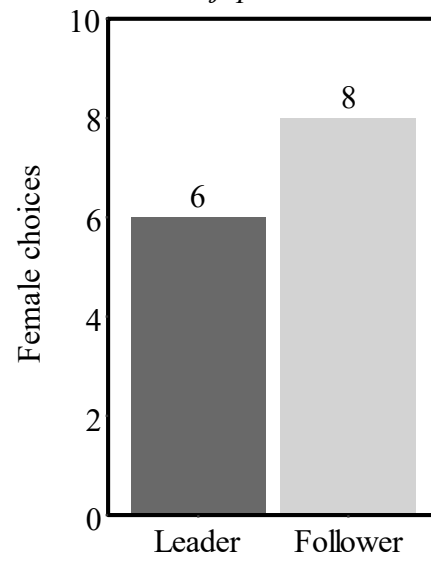


Figure 5.5. Female *B. japonica* preference for leading or following calls. Each female choice represents a single female.

5.6 References

- Backwell, P., Jennions, M., Passmore, N., and Christy, J. (1998). Synchronized courtship in fiddler crabs. *Nature* 391: 31-32.
- Backwell, P., Jennions, M., Wada, K., Murai, M., and Christy, J. (2006). Synchronous waving in two species of fiddler crabs. *Acta Ethologica* 9: 22-25.
- Bee, M. A. (2007). Selective phonotaxis by male wood frogs (*Rana sylvatica*) to the sound of a chorus. *Behavioral Ecology and Sociobiology* 61: 955-966.
- Bee, M. A., and Swanson, E. M. (2007). Auditory masking of anuran advertisement calls by road traffic noise. *Animal Behaviour* 74: 1765-1776.
- Bernal, X. E., and Pinto, C. M. (2016). Sexual differences in prevalence of a new species of trypanosome infecting túngara frogs. *International Journal for Parasitology: Parasites and Wildlife* 5:40-47.
- Borkent, A. (2008). The frog-biting midges of the world (Corethrellidae: Diptera). *Zootaxa* 1804: 1-456.
- Bosch, J., and Márquez, R. (2002). Female preference function related to precedence effect in an amphibian anuran (*Alytes cisternasii*): tests with non-overlapping calls. *Behavioral Ecology* 13: 149-153.
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., and Bolker, B. M. (2017). *glmmTMB* balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* 9: 378-400.
- Brown, A. D., Stecker, G. C., and Tollin, D. J. (2015). The precedence effect in sound localization. *Journal of the Association for Research in Otolaryngology* 16: 1-28.
- Brumm, H., and Slabbekoorn, H. (2005). Acoustic communication in noise. *Advances in the Study of Behavior* 35: 151-209.
- Buck, J., and Buck, E. (1966). Biology of synchronous flashing of fireflies. *Nature* 211: 562-564.
- Buck, J., and Buck, E. (1978). Toward a functional interpretation of synchronous flashing by fireflies. *The American Naturalist* 112: 471-492.
- Camp, J. V. (2006). *Host attraction and host selection in the family Corethrellidae (Wood and Borkent)(Diptera)*. Unpublished master's thesis. Georgia Southern University, Statesboro.
- Camp, J. V., Bakonyi, T., Soltész, Z., Zechmeister, T., and Nowotny, N. (2018). *Uranotaenia unguiculata* Edwards, 1913 are attracted to sound, feed on amphibians, and are infected with multiple viruses. *Parasites and Vectors* 11: 456.
- Capshaw, G., Foss-Grant, A. P., Hartmann, K., Sehuanes, J. F., and Moss, C. F. (2020). Timing of the advertisement call of the common tink frog (*Diasporus diastema*) shifts with the acoustic behaviour of local conspecifics. *Bioacoustics* 29: 79-96.
- Carlson, A. D., and Copeland, J. (1985). Flash communication in fireflies. *The Quarterly review of biology* 60: 415-436.
- Chen, T. C., Kam, Y. C., and Lin, Y. S. (2001). Thermal physiology and reproductive phenology of *Buergeria japonica* (Rhacophoridae) breeding in a stream and a geothermal hot spring in Taiwan. *Zoological Science* 18: 591-596.
- Clulow, S., Mahony, M., Elliott, L., Humfeld, S., and Gerhardt, H. C. (2017). Near-synchronous calling in the hip-pocket frog *Assa darlingtoni*. *Bioacoustics* 26: 249-258.
- de Soto, N. A., Visser, F., Tyack, P. L., Alcazar, J., Ruxton, G., Arranz, P., Madsen, P. T., and Johnson, M. (2020). Fear of Killer Whales Drives Extreme Synchrony in Deep Diving Beaked Whales. *Scientific Reports* 10: 1-9.

- Dent, M. L., and Dooling, R. J. (2004). The precedence effect in three species of birds (*Melopsittacus undulatus*, *Serinus canaria*, and *Taeniopygia guttata*). *Journal of Comparative Psychology* 118: 325-331.
- Duellman, W. E. (1970). *Hylid frogs of Middle America*. University of Kansas Press, Lawrence.
- Dyson, M. L. and N. I. Passmore. 1988. Two-choice phonotaxis in *Hyperolius marmoratus* (Anura: Hyperoliidae): the effect of temporal variation in presented stimuli. *Animal Behaviour* 36: 648-652.
- Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *The American Naturalist* 139: S125-S153.
- Farabaugh, S. M. (1982). The ecological and social significance of duetting. In *Acoustic communication in birds, Vol. 2 Song learning and its consequences*, (eds. D. E. Kroodsma and E. H. Miller) pp. 85-124. Academic Press, New York.
- Faul, F., Erdfelder, E., Buchner, A., and Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods* 41: 1149-1160.
- Gerhardt H.C., and Klump G.M. (1988). Masking of acoustic signals by the chorus background noise in the green tree frog: A limitation on mate choice. *Animal Behaviour* 36: 1247-1249.
- Gerhardt, H. C. (1975). Sound pressure levels and radiation patterns of the vocalizations of some North American frogs and toads. *Journal of Comparative Physiology* 102: 1-12.
- Gerhardt, H. C. (1987). Evolutionary and neurobiological implications of selective phonotaxis in the green treefrog, *Hyla cinerea*. *Animal Behaviour* 35: 1479-1489.
- Gerhardt, H. C., and Huber, F. (2002). *Acoustic communication in insects and anurans: common problems and diverse solutions*. University of Chicago Press, Chicago.
- Grafe, T. U. (1996). The function of call alternation in the African reed frog (*Hyperolius marmoratus*): precise call timing prevents auditory masking. *Behavioral Ecology and Sociobiology* 38: 149-158.
- Grafe, T. U. (1999). A function of synchronous calling and a novel female preference shift in an anuran. *Proceedings of the Royal Society B* 266: 2331-2336.
- Grafe, T. U. (2003). Synchronized interdigitated calling in the Kuvangu running frog, *Kassina kuvangensis*. *Animal Behaviour* 66: 127-136.
- Greenfield, M. D. (1994). Synchronous and alternating choruses in insects and anurans: common mechanisms and diverse functions. *American Zoologist* 34: 605-615.
- Greenfield, M. D., and Roizen, I. (1993). Katydid synchronous chorusing is an evolutionarily stable outcome of female choice. *Nature* 364: 618-620.
- Greenfield, M. D., and Schul, J. (2008). Mechanisms and evolution of synchronous chorusing: emergent properties and adaptive functions in *Neoconocephalus* katydids (Orthoptera: Tettigoniidae). *Journal of Comparative Psychology* 122: 289-297.
- Greenfield, M. D., Tourtellot, M. K., and Snedden, W. A. (1997). Precedence effects and the evolution of chorusing. *Proceedings of the Royal Society B* 264: 1355-1361.
- Hall, M. L. (2004). A review of hypotheses for the functions of avian duetting. *Behavioral Ecology and Sociobiology* 55: 415-430.
- Harrington, F. H. (1989). Chorus howling by wolves: acoustic structure, pack size and the Beau Geste effect. *Bioacoustics* 2: 117-136.

- Hartbauer, M., Kratzer, S., Steiner, K., and Römer, H. (2005). Mechanisms for synchrony and alternation in song interactions of the bushcricket *Mecopoda elongata* (Tettigoniidae: Orthoptera). *Journal of Comparative Physiology A* 191: 175-188.
- Höbel, G. (2010). Interaction between signal timing and signal feature preferences: causes and implications for sexual selection. *Animal Behaviour* 79: 1257-1266.
- Hothorn T, Hornik K, van de Wiel MA, Zeileis A (2006). "A Lego system for conditional inference." *The American Statistician* 60: 257–263.
- Howard, R. D., and Palmer, J. G. (1995). Female choice in *Bufo americanus*: effects of dominant frequency and call order. *Copeia* 1995: 212-217.
- Humphreys, R. K., and Ruxton, G. D. (2017). Consequences of grouped data for testing for departure from circular uniformity. *Behavioral Ecology and Sociobiology* 71: 167.
- Ibáñez, R. (1993). Female phonotaxis and call overlap in the neotropical glassfrog *Centrolenella granulosa*. *Copeia* 1993: 846-850.
- Johnson, R. N., Young, D. G., and Butler, J. F. (1993). Trypanosome transmission by *Corethrella wirthi* (Diptera: Chaoboridae) to the green treefrog, *Hyla cinerea* (Anura: Hylidae). *Journal of Medical Entomology* 30: 918-921.
- Kime, N. M., Turner, W. R., and Ryan, M. J. (2000). The transmission of advertisement calls in Central American frogs. *Behavioral Ecology* 11: 71-83.
- Klump, G. M., and Gerhardt, H. C. (1992). Mechanisms and function of call-timing in male-male interactions in frogs. In *Playback and studies of animal communication*. (ed. P. K. McGregor), pp. 153–174. Plenum Press, New York.
- Kokko, H., Sutherland, W. J., Lindstrom, J., Reynolds, J. D., and Mackenzie, A. (1998). Individual mating success, lek stability, and the neglected limitations of statistical power. *Animal Behaviour* 56: 755-762.
- Kuramoto, M. (1986). Call structures of the rhacophorid frogs from Taiwan. *Scientific report of the Laboratory for Amphibian Biology, Hiroshima University* 8: 45-68.
- Lee, N., Elias, D. O., and Mason, A. C. (2009). A precedence effect resolves phantom sound source illusions in the parasitoid fly *Ormia ochracea*. *Proceedings of the National Academy of Sciences* 106: 6357-6362.
- Legett, H. D., Aihara, I., and Bernal, X. E. (2020). Signal Synchrony and Alternation Among Neighbor Males in a Japanese Stream Breeding Treefrog, *Buergeria japonica*. *Current Herpetology*, 39: 80-85.
- Legett, H. D., Hemingway, C. T., and Bernal, X. E. (in press). Prey exploits the auditory illusions of eavesdropping predators. *The American Naturalist*, 195.
- Legett, H. D., Page, R. A., and Bernal, X. E. (2019). Synchronized mating signals in a communication network: the challenge of avoiding predators while attracting mates. *Proceedings of the Royal Society B*, 286: 20191067.
- Lenth, R., Singmann, H., and Love, J. (2018). *Emmeans*: Estimated marginal means, aka least-squares means. R package version 1.4.5.
- Lund U., and Agostinelli C. (2018). *CircStats*: circular statistics. R package version 0.2-6.
- Marshall, V. T. and H. C. Gerhardt. 2010. A precedence effect underlies preferences for calls with leading pulses in the grey treefrog, *Hyla versicolor*. *Animal Behavior* 80:139-145.
- McCarley, H. (1975). Long-distance vocalizations of coyotes (*Canis latrans*). *Journal of Mammalogy* 56: 847-856.
- McGregor, P. K., and Peake, T. M. (2000). Communication networks: social environments for receiving and signalling behaviour. *Acta Ethologica* 2: 71-81.

- McKeever, S., and Hartberg, W. K. (1980). An effective method for trapping adult female *Corethrella* (Diptera: Chaoboridae). *Mosquito News* 20: 111-112.
- Miyagi, I., and Toma, T. (2013). *Uranotaenia* (Pseudoficalbia) *tanakai* (Diptera, Culicidae), a new species from forest swamps, Iriomote Is., the Ryukyu Archipelago, Japan. *Medical Entomology and Zoology* 64: 167-174.
- Moiseff, A., and Copeland, J. (2010). Firefly synchrony: a behavioral strategy to minimize visual clutter. *Science* 329: 181-181.
- Morin, J. G. (1986). Firefleas of the sea: luminescent signaling in marine ostracode crustaceans. *Florida Entomologist* 69: 105-121.
- Nityananda, V., and Balakrishnan, R. (2007). Synchrony during acoustic interactions in the bushcricket Mecopoda ‘Chirper’ (Tettigoniidae: Orthoptera) is generated by a combination of chirp-by-chirp resetting and change in intrinsic chirp rate. *Journal of Comparative Physiology A* 193: 51.
- Penna, M., and Solís, R. (1998). Frog call intensities and sound propagation in the South American temperate forest region. *Behavioral Ecology and Sociobiology* 42: 371-381.
- R Core Team (2013). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Reaney, L. T., Sims, R. A., Sims, S. W., Jennions, M. D., and Backwell, P. R. (2008). Experiments with robots explain synchronized courtship in fiddler crabs. *Current Biology* 18: R62-R63.
- Reichert, M. S., L. B. Symes, and G. Höbel. (2016). Lighting up sound preferences: cross-modal influences on the precedence effect in treefrogs. *Animal Behaviour* 119: 151-159.
- Ryan, M. J. (1985). *The túngara frog: a study in sexual selection and communication*. University of Chicago Press, Chicago.
- Ryan, M. J. (1986). Synchronized calling in a treefrog (*Smilisca sila*). *Brain, Behavior and Evolution* 29: 196-206.
- Ryan, M. J., and Keddy-Hector, A. (1992). Directional patterns of female mate choice and the role of sensory biases. *The American Naturalist* 139: S4-S35.
- Ryan, M. J., and Rand, A. S. (1990). The sensory basis of sexual selection for complex calls in the túngara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). *Evolution* 44: 305-314.
- Ryan, M. J., Tuttle, M. D., and Taft, L. K. (1981). The costs and benefits of frog chorusing behavior. *Behavioral Ecology and Sociobiology* 8: 273-278.
- Schul, J., Bush, S. L., and Frederick, K. H. (2014). Evolution of call patterns and pattern recognition mechanisms in *Neoconocephalus* katydids. In *Insect hearing and acoustic communication*. (ed. B. Hedwig). pp. 167-183. Springer, Berlin.
- Schwartz, J. J. (1986). Male calling behavior and female choice in the neotropical treefrog *Hyla microcephala*. *Ethology* 73: 116-127.
- Shaw, K. C., Galliard, P. L., and Smith, B. (1990). Acoustic behavior of *Amblycorypha parvipennis* (Orthoptera: Tettigoniidae). *Annals of the Entomological Society of America* 83: 617-625.
- Shelly, T. E., and Greenfield, M. D. (1991). Dominions and desert clickers (Orthoptera: Acrididae): influences of resources and male signaling on female settlement patterns. *Behavioral Ecology and Sociobiology* 28: 133-140.
- Sismondo, E. (1990). Synchronous, alternating, and phase-locked stridulation by a tropical katydid. *Science* 249: 55-58.

- Tang, W. (2009). *The chorus sound characteristics and phonotactic behavior of Buergeria japonica*. Unpublished master's thesis. National Cheng Kung University, Tainan City.
- Toma, T., Miyagi, I., and Tamashiro, M. (2014). Blood meal identification and feeding habits of *Uranotaenia* species collected in the Ryukyu Archipelago. *Journal of the American Mosquito Control Association* 30: 215-218.
- Toma, T., Takara, T., Miyagi, I., Futami, K., and Higa, Y. (2019). Mosquitoes and frog-biting midges (Diptera: Culicidae and Corethrellidae) attracted to traps with natural frog calls and synthesized sounds at Iriomote Island, Ryukyu Archipelago, Japan. *Medical Entomology and Zoology* 70: 221-234.
- Tuttle, M. D., and Ryan, M. J. (1982). The role of synchronized calling, ambient light, and ambient noise, in anti-bat-predator behavior of a treefrog. *Behavioral Ecology and Sociobiology* 11: 125-131.
- Walker, T. J. (1969). Acoustic synchrony: two mechanisms in the snowy tree cricket. *Science* 166: 891-894.
- Wallach, H., E. B. Newman, and M. R. Rosenzweig. 1949. A precedence effect in sound localization. *The Journal of the Acoustical Society of America* 21: 468-468.
- Wang, Y. H., Hsiao, Y. W., Lee, K. H., Tseng, H. Y., Lin, Y. P., Komaki, S., and Lin, S. M. (2017). Acoustic differentiation and behavioral response reveals cryptic species within *Buergeria* treefrogs (Anura, Rhacophoridae) from Taiwan. *PloS one* 12: e0184005.
- Wells, K. D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour* 25: 666-693.
- Wells, K. D., and Schwartz, J. J. (1984). Vocal communication in a neotropical treefrog, *Hyla ebraccata*: advertisement calls. *Animal Behaviour* 32: 405-420.
- Whitney, C. L., and Krebs, J. R. (1975). Mate selection in Pacific tree frogs. *Nature* 255: 325-326.
- Wickler, W., and Seibt, U. (1974). Rufen und Antworten bei *Kassina senegalensis*, *Bufo regularis* und anderen Anuren. *Zeitschrift fur Tierpsychologie* 34: 524-537.
- Wollerman, L. (1999). Acoustic interference limits call detection in a Neotropical frog *Hyla ebraccata*. *Animal Behaviour* 57: 529-536.
- Wollerman, L., and Wiley, H. R. (2002a). Possibilities for error during communication by neotropical frogs in a complex acoustic environment. *Behavioral Ecology and Sociobiology* 52: 465-473.
- Wollerman, L., and Wiley, R. H. (2002b). Background noise from a natural chorus alters female discrimination of male calls in a Neotropical frog. *Animal Behaviour* 63: 15-22.
- Wytenbach, R. A., and Hoy, R. R. (1993). Demonstration of the precedence effect in an insect. *The Journal of the Acoustical Society of America* 94: 777-784.
- Zuk, M., and Kolluru, G. R. (1998). Exploitation of sexual signals by predators and parasitoids. *The Quarterly Review of Biology* 73: 415-438.

CONCLUSION

Animals in mating aggregations can benefit in multiple ways by synchronizing their mating signals. Synchronized signals can reduce the attraction of eavesdropping predators at multiple scales, both between aggregations and within an aggregation (the eavesdropper avoidance hypothesis). In addition, synchronized signals can constructively interfere, increasing the peak amplitude of the combined signals and attracting mates from a greater distance (the beacon effect hypothesis). In Chapter 1, I found that eavesdropping frog-eating bats (*Trachops cirrhosus*) and frog-biting midges (*Corethrella* spp.) prefer unsynchronized frog calls to synchronized calls in two neotropical anuran species (*S. sila* and *E. pustulosus*). In Chapter 2, I found that bats and midges also have strong preferences for leading over following synchronized frog calls. In Chapter 3, I described a unique case in which male tree frogs (*B. japonica*) produce both alternating calls and near-complete overlapped synchronized calls. In Chapter 4, I found that eavesdropping midges (*C. nippon*) and mosquitoes (*Uranotaenia* spp.) prefer the alternating *B. japonica* call type over the synchronized call type when both call types were presented unsynchronized. In addition, the overall rates of eavesdropper attraction were notably lower for *B. japonica* compared to *S. sila* and *E. pustulosus* in the neotropics, suggesting a minimal benefit from evading eavesdroppers in this system. In Chapter 5, I found that midges and mosquitoes prefer leading over following synchronized *B. japonica* calls. Synchronized calls were also more attractive to female *B. japonica* at distance from the chorus compared to unsynchronized calls.

In both synchronous species examined in this dissertation, *S. sila* and *B. japonica*, female frogs display reduced preferences for fine-scale signal timings. It is the preference for unmasked signals that is assumed to drive the use of non-synchronous signaling as a general strategy (Wollerman and Wiley 2002a, Wollerman and Wiley 2002b). Female *S. sila* have a reduced preference for unsynchronized calls and a reduced leading-following call preference. Female *B. japonica* also display a reduced leading-following call preference and actually prefer sets of synchronized calls to unsynchronized calls. Reduced signal timing preferences in females may be a commonality among synchronizing species. In addition to *S. sila* and *B. japonica*, the females of other anuran species that synchronized their calls also have no leading-following call

preferences (*Centrolenella granulosa*: Ibáñez 1993) or reversed leading-following call preferences (*Dendropsophus ebraccata*: Wells and Schwartz 1984; *Kassina fusca*: Grafe 1999). For these synchronizing species, the selective pressure from females against producing synchronized calls is relaxed. Thus, while synchronized mating signals may provide multiple benefits, and these benefits may be widely enjoyed by signaling males across species, it is relaxed selection from females (relaxed sexual selection hypothesis) that provides males the opportunity to synchronize their mating signals. Reduced selective pressure shifts the tradeoffs between costs and benefits of traits, ultimately resulting in trait evolution. Previously, however, the concept of relaxed selection has only been applied in non-sexual selective contexts, such as predator release or shifts in environmental regimes (Lahti 2009). Results from the research detailed in this dissertation suggest that relaxed female preferences allow for the evolution and maintenance of synchronized mating signals. Similar shifts in preference are expected across synchronized signaling organisms and signal modalities.

Overall, mating signal synchronization remains a relatively unexplored phenomenon. The foremost obstacle for the study of this topic is that fine-scale signal timings are often not reported with other aspects of signaling behavior, such as signal duration and dominant frequency (e.g. Duellman 1970). Previous studies of *B. japonica* signaling behavior, for example, do not reference call synchronization (Kuramoto 1986; Tang 2009; Wang et al. 2017). Thus, there is a need for further natural history studies, especially in species where synchronization has been suggested but has not been described and measured (*Anaxyrus americanus*: Wells 1977; *Hyla arenicolor*: Marshall and Gerhardt unpublished data discussed in Gerhardt and Huber 2002).

There are also further evolutionary and mechanistic questions still to be explored following this dissertation. I found that synchronization can affect both female and eavesdropper attraction. However, this was only examined at a limited categorical scale: synchronized-unsynchronized or leading-following calls within a synchronized set. Signal synchronization occurs along a continuum, ranging from two signals abutting to being 100 % overlapped. It is likely that both female and eavesdropper preferences for synchronization change as a function of that degree of signal overlap. Relatedly, the relative timing of signal production between neighboring males in a chorus is likely not static. Signaling males may plastically alter the latency of signal production

in response to their social environment or perceived predation risk, similar to other calling behaviors (Page and Ryan 2005). Thus, further behavioral studies are needed to assess female and eavesdropper preferences for synchronized signals along a gradient of signal latencies and to examine the plasticity of signal overlap between males in a chorus. Finally, reduced leading-following signal preferences in female receivers may be a commonality among species that synchronize their mating signals. Yet, how females of these species overcome the precedence effect is still a mystery. Further studies that examine the auditory physiologies of synchronizing species and their non-synchronous congeners are needed to understand the mechanisms underlying the use of synchronized mating signals.

References

- Duellman, W. E. (1970). *Hylid frogs of Middle America*. University of Kansas Press, Lawrence.
- Gerhardt, H. C., and Huber, F. (2002). *Acoustic communication in insects and anurans: common problems and diverse solutions*. University of Chicago Press, Chicago.
- Grafe, T. U. (1999). A function of synchronous calling and a novel female preference shift in an anuran. *Proceedings of the Royal Society B* 266: 2331–2336.
- Ibáñez, R. (1993). Female phonotaxis and call overlap in the neotropical glassfrog *Centrolenella granulosa*. *Copeia* 1993: 846-850.
- Kuramoto, M. (1986). Call structures of the rhacophorid frogs from Taiwan. *Scientific report of the Laboratory for Amphibian Biology, Hiroshima University* 8: 45-68.
- Lahti, D. C., Johnson, N. A., Ajie, B. C., Otto, S. P., Hendry, A. P., Blumstein, D. T., Cross, R. G., Donohue, K., and Foster, S. A. (2009). Relaxed selection in the wild. *Trends in ecology and Evolution* 24: 487-496.
- Page, R. A. and Ryan, M. J. (2005). Flexibility in assessment of prey cues: frog-eating bats and frog calls. *Proceedings of the Royal Society B* 272: 841-847.
- Tang, W. (2009). *The chorus sound characteristics and phonotactic behavior of Buergeria japonica*. Unpublished master's thesis. National Cheng Kung University, Tainan City.
- Wang, Y. H., Hsiao, Y. W., Lee, K. H., Tseng, H. Y., Lin, Y. P., Komaki, S., and Lin, S. M. (2017). Acoustic differentiation and behavioral response reveals cryptic species within Buergeria treefrogs (Anura, Rhacophoridae) from Taiwan. *PloS one* 12: e0184005.
- Wells, K. D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour* 25: 666-693.
- Wells, K. D., and Schwartz, J. J. (1984). Vocal communication in a neotropical treefrog, *Hyla ebraccata*: advertisement calls. *Animal Behaviour* 32: 405-420.
- Wollerman, L., and Wiley, H. R. (2002a). Possibilities for error during communication by neotropical frogs in a complex acoustic environment. *Behavioral Ecology and Sociobiology* 52: 465-473.
- Wollerman, L., and Wiley, R. H. (2002b). Background noise from a natural chorus alters female discrimination of male calls in a Neotropical frog. *Animal Behaviour* 63: 15-22.