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Sources of Variation in Social Plasticity in Female Mate Preferences and Male Traits

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SOURCES OF VARIATION IN SOCIAL PLASTICITY IN FEMALE MATE PREFERENCES AND

MALE TRAITS

by

Daniel Neelon

A Dissertation Submitted in

Partial Fulfillment of the

Requirements for the Degree of

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ABSTRACT
SOURCES OF VARIATION IN SOCIAL PLASTICITY IN FEMALE MATE PREFERENCES AND
MALE TRAITS

by

Daniel Neelon

University of Wisconsin-Milwaukee, 2018
Under the Supervision of Professor Gerlinde Höbel

Female preferences are an important cause of selection on male traits, and exploring

the sources of variation in these preferences has been the focus of intense behavioral

research. This is due to the fact that understanding this variation is integral to understating

the maintenance of variation and complexity in male traits, as well as the evolution and

divergence of populations. This project aims to explore the sources of variation in these

mating preferences, as well as examine whether similar sources affect components of male

traits. This was accomplished using the well-studied mating system of the Green Treefrog,

Hyla cinerea that features female preferences for the male advertisement call. Using

playback experiments, I dissect the constituent parts of female preferences – preference

functions and choosiness, and demonstrate that sources of variation in preferences can be

due to interactions between choosiness and social experience, as well as interactions

between female body size and the shape of a female's preference function and the

constituent traits of the preference function curve. Further, I demonstrate that social

experience also affects the male trait, and can drive variation in the presentation of

advertisement calls. This work demonstrates that while sexual selection can be a strong

and directional driver of the evolution of mating behaviors, substantial variation can arise

from a number of sources that may have profound effects for the evolution of species.

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ACKNOWLEDGEMENTS

I'd like to thank my committee: Dr. Gerlinde Höbel, Dr. Rafa Rodrìguez, Dr. Linda Whittingham, Dr. Emily Latch, and Dr. Peter Dunn. I extend my most heartfelt gratitude to my advisor, Dr. Gerlinde Höbel, for her invaluable assistance and guidance, and for sharing her knowledge of all things froggish with me. It was only through her unrelenting support and patience that this project came to fruition.   I'd also like to thank my field assistants - Jessica Grow, Kassi Mohamed, Christopher Lang - and fellow lab mates who assisted me in data collection and analysis, Finally I'd like to thank my mother, my Aunt Mary Jo, and my entire family for supporting me on this journey of discovery.

Introduction

Female preferences are an important cause of selection on male sexual traits (Andersson 1994). Uniformity in female preferences was previously believed to be necessary for the evolution of extreme ornaments or behaviors (Mead and Arnold 2004), however the existence of substantial variation in female preferences has been of great interest for some time (Cotton et al. 2006; Bakker 1999; Hunt 2005; Byers et al. 2006; Uetz and Norton 2007; Holveck et al. 2011; DuVal and Kapoor 2015). Elucidating the causes of this variation in has been the focus of intense behavioral research, due to the fact that understanding this variation in female preferences is integral to understating the maintenance of variation and complexity in male traits, as well as the evolution and divergence of populations. (Servedio et al. 2009; Hebets and Sullivan-Beckers 2010; Svensson et al. 2010; Rodríguez et al. 2013; Rebar and Rodríguez 2016; Fowler-Finn et al. 2017). This project aims to explore the sources of variation in female mating preferences, as well as examine whether similar sources affect components of male traits, using the well-studied mating system of the Green Treefrog, *Hyla cinerea*. (Gerhardt 1974; Gerhardt et al. 1987, Höbel and Gerhardt 2003)

Chapter 1 focuses on one aspect of female preferences, choosiness, and explores how social experience can drive it's variation, which ultimately has serious implications for mate choice decisions. Chapter 2 broadens the scope to ask whether variation in choosiness is inextricably linked to a female's preference function, or are the two independent components of mate choice, that can vary independently. Finally, Chapter 3 focuses on whether similar factors that drive variation in female preferences also affect the traits of the male advertisement call, particularly during intrasexual interactions.

Social plasticity in choosiness in green tree frogs, Hyla cinerea

Abstract

Mate choice is an important driver of the evolution of sexual traits and can promote divergence and speciation. Understanding the underlying variation in mate choice decisions is crucial to understand variation in the strength and direction of sexual selection. We explored whether variation in the social environment influences mate choice decisions and focus on the aspect of mate choice termed choosiness (i.e. the effort invested in mate assessment and acquisition). Using call playbacks, we manipulated the social environment female green tree frogs would experience as they entered a chorus, and then we conducted two-choice playback trials to assess whether females exhibited social plasticity in choosiness. We explored social plasticity at 2 levels: in one experiment, we manipulated the presence or absence of preferred (attractive) and less preferred (unattractive) conspecific males (i.e. intraspecific context), and in the other experiment, we manipulated the presence or absence of preferred (conspecific) and less preferred closely related heterospecific males (i.e. interspecific context). We found that in the intraspecific context, the presence of attractive males increased choosiness, while absence of attractive males reduced choosiness. In the interspecific context, choosiness remained stable in most treatments, but was lowered when females experienced a mixture of conspecific and heterospecific calls. We discuss the effect of social plasticity in choosiness on mate choice decisions and highlight its evolutionary consequences.

INTRODUCTION

Mate choice is an important cause of sexual selection, driving the evolution of phenomena

such as extravagant sexual ornaments and elaborate mating displays (Darwin 1871; West-

Eberhard 1983; Andersson 1994; Hoekstra et al. 2001; Kingsolver et al. 2001). Mate choice

can also promote speciation events via the rapid divergence of sexual traits and the

reproductive isolation that this generates (Fisher 1958; West-Eberhard 1983, 2014; Coyne

and Orr 2005; Svensson 2006; Seddon et al. 2008; 2013). Consequently, understanding

variation in the strength and direction of sexual selection (and the resulting effects on

sexual trait diversity and speciation) requires assessing the causes and consequences of

variation in mate choice decisions between individuals (Jennions and Petrie 1997; Coleman

et al. 2004).

Mate choice decisions vary among individuals for a number of reasons, including

such diverse elements as the age, condition, or reproductive stage of the choosing

individual (Cotton et al. 2006; Bakker 1999; Hunt 2005; Byers et al. 2006; Uetz and Norton

2007; Holveck et al. 2011; DuVal and Kapoor 2015). Recent work has also highlighted the

contribution of social plasticity — i.e., variation arising from the social environment. The

reason for this focus is that mate choice decisions, as behaviors in general, are highly

plastic and incredibly responsive to the variation in social conditions (Rosenqvist 1997;

Bailey 2011; Foster 2013; Snell-Rood 2013; Zuk et al. 2014). This plasticity generates

ongoing evolutionary feedback loops, because the cause of selection (the social

environment) coevolves with the targets of selection (the signals and preferences of

individuals), which then go on to constitute the social environment in the next generation

(West-Eberhard, 1983, 2014). These feedback loops influence the form and strength of

sexual selection, and depending on the type of response, also influence whether mate choice dynamics promote the maintenance of variation or divergence (Servedio et al. 2009; Hebets and Sullivan-Beckers 2010; Svensson et al. 2010; Rodríguez et al. 2013; Rebar and Rodríguez 2016; Fowler-Finn et al. 2017)

Analysis of social plasticity in mate choice decisions can be approached in terms of its two main components: "mate preference functions" (the relative ranking of the attractiveness of perspective mates) and "choosiness" (the effort invested in mate assessment) (Jennions and Petrie 1997). There is substantial evidence for social plasticity in mate preferences (reviewed in Rodríguez et al. 2013; Fowler-Finn et al. 2017), while social plasticity in choosiness has received less attention (but see Lindstrom and Lehtonen 2013).

Here we explore social plasticity in choosiness in green tree frogs, *Hyla cinerea*. Anuran choruses are incredibly dynamic and complex, varying in the composition of attractive and unattractive mates as well as in the assemblage of species present (Gerhardt et al. 1987; Conant and Collins 1998). Females are challenged to choose among, and then travel to, calling males that range from preferred (attractive conspecifics) to less preferred (unattractive conspecifics or unsuitable heterospecifics). This cacophony of male calls constituting the chorus provides females with information about the current presence of male mate types and may generate variation in choosiness among females.

We tested the hypothesis that social plasticity in choosiness influences mate choice decisions. We exposed female green tree frogs to treatments of acoustic experience representing variation in chorus composition. We conducted two experiments that

manipulated the social environment at different levels: (i) within-species variation (conspecific level) and (ii) between-species variation (heterospecific level). With these treatments, the social plasticity hypothesis predicts that females exposed to different social environments will vary in choosiness for preferred males. Note, however, that there is no a priori expectation that behavioral changes resulting from social plasticity have to be adaptive. In response to within-species variation, social plasticity has likely been under selection, making an adaptive function more plausible. In this case, the above hypothesis can be refined to predict that females that experience attractive calls will be more choosy than females that experience unattractive ones, or no calls at all, because it assures that females only invest in mate sampling if it is worthwhile (i.e., if there are attractive males present). By contrast, whether social plasticity in response to between-species variation has been under selection for an adaptive function will depend on the level of sympatry with heterospecifics, the local abundance of conspecifics and heterospecifics, and the opportunity and cost of mating with heterospecifics. While about half of the range of green treefrogs overlaps with the heterospecific used in our experiments, the green treefrog population under study here is allopatric, i.e., has no evolutionary history with this heterospecific (Conant and Collins 1998). It is therefore difficult to predict how females would respond to them. If social plasticity in response to between-species variation shows a similar pattern than the one expected in response to within-species variation, we would predict that females that experience conspecific calls will be more choosy than females that experience heterospecific ones, or no calls at all. This would assure that females invest more in mate sampling if it is worthwhile (i.e., if there are conspecific males present).

METHODS AND MATERIALS

Study Species and Study Site

Green tree frogs, *Hyla cinerea*, (Anura: Hylidae), are a common anuran species found throughout the southeastern United States (Conant and Collins 1998). Each night from April through July, males congregate near ponds and swampy areas, creating large choruses where they advertise for females for two to four hours. Our focal population breeds in ponds at the East Texas Conservation Center, in Jasper, TX, where we performed all trials during May-July of 2012 and 2013. To minimize social experience with the local acoustic environment, we collected females during the first hour after the breeding chorus formed, and then conducted the experience and testing phases of the experiments during the rest of the night. All females were collected from amplexed pairs to ensure sexual receptivity, and all frogs were released at the site of capture after the conclusion of the night's trials. Experimental procedures were approved by the Animal Care and Use Committee of the University of Wisconsin-Milwaukee (IACUC 07-08#38).

General Experimental Design

Call Stimulus Generation: All stimuli used in the experiment were synthetic call stimuli modeled after the respective species' advertisement call (for specific call trait values, see Experiment 1 and Experiment 2 below). We generated the synthetic stimuli using a custom-written DOS program (courtesy of J.J. Schwartz), and then used sound editing software (Audacity 2.0.0. 2012) to control stimulus timing and create audio files for use in the experience and testing phase of the experiments.

To simulate chorus environments for the experience phase, we created three sound files per experiment corresponding to three experience treatments: preferred, non-preferred, and mixed (the silent treatments did not require call playback). In both experiments, for the preferred and non-preferred experience treatments, we repeatedly pasted a call stimulus, separated by periods of silence, to make longer call sequences. The duration of the inter-call intervals was varied randomly (but within the range of the respective species) to simulate variability in call periods of several interacting males. To generate the mixed experience treatments, we replaced every other preferred stimulus with a non-preferred stimulus, resulting in a 1:1 ratio of call types.

To assess choosiness during the testing phase, we used the same call stimuli as those generated for the experience treatments to conduct two-choice playback trials. We used Audacity software to create stereo files that played a preferred call stimulus on one channel, and a less preferred call stimulus on the other channel. Stimuli were set to exactly alternate with each other. The stimulus period was set to 800 ms (per channel), which is within the range of call period variation of either species.

Experience Phase: Females experienced treatments of simulated chorus environments during an experience phase lasting 3 hours. This time frame reasonably represents the length of time during which a female could assess a male chorus. In Experiment 1 we manipulated experience within the range of variation of conspecific males, and in Experiment 2 we manipulated experience with the presence or absence of conspecific versus closely related heterospecific males (see specific experimental methods

below) In both experiments, we assigned females to the experiments and treatments randomly.

During the experience phases of each experiment, females were placed in groups of two to five in a mesh screen cage (Exo Terra Explorarium; 45x60cm). These cages were placed into quiet areas of the study site (i.e., away from active frog choruses). Treatment stimuli were broadcast from a MP3 player (RCA TH2002RDR) through a iHome rechargeable mini speaker (iHM60) placed next to each cage. Playbacks were adjusted to a sound pressure level of 73dB, which is representative of natural chorus noise (Velez et al 2012).

Testing Phase: Immediately after the conclusion of the experience treatments, we assessed choosiness with two-choice playback trials. Our assay of choosiness is designed to represent the distance over which a female is willing to perform phonotaxis in order to reach a mate that she finds attractive. We used stimulus amplitude as a stand-in for distance, taking advantage of the inverse square law of sound attenuation: with each doubling of the distance to the sound source the stimulus amplitude decreases by 6 dB (Speaks 1997). The basis for our assay is that for green tree frogs (as for many anurans), call preferences are amplitude dependent, i.e., it is possible to reverse a preference by changing the relative amplitude in favor of the other stimulus (e.g., Gerhardt 1982; 1987, Höbel and Gerhardt 2003). We used this amplitude-dependence of preferences to determine how "choosy" each female was. Specifically, our measure of choosiness was the greatest amplitude difference (in rel. dB) at which the female still approached the preferred stimulus. For example, a female that preferred stimulus A over stimulus B when stimuli are

broadcast favoring B by 0, 3, 6, and 9 dB, respectively, but started to approach B once the

amplitude difference reached 12 dB, would be given a choosiness score of 9 dB (i.e.,

greatest amplitude difference she still approached her preferred call). Thus, our measure of

choosiness follows Jennions and Petrie (1997), modified to represent the effort a female

expends in securing her preferred male. This may entail the actual cost of locomotion, plus

additional risks such as the chance of encountering predators, which increases with the

time and distance the female covers. Nota bene: we have evidence that this measure of

choosiness represents a trait that varies independently from mate preference functions (D.

Neelon and G. Höbel, in prep).

We tested all females in an outdoor playback arena set up in a dark location at our

study site. By the time we started the choice trials, chorus activity had already ceased for

the night, assuring quiet testing conditions. The arena was 2m long and 1m wide, consisting

of a plywood floor, and a wood frame 50cm in height, which was screened with a visually

opaque but acoustically transparent black cloth. Two speakers (JBL Control 1X) were

placed opposite one another along the central long axis of the arena, just outside of the

screen (i.e., females could hear the stimuli, but not see the speakers). Stimuli were

broadcast from a PC laptop using Audacity software (version 2.0.0). The female was placed

in a 10 cm wire cage in the center of the arena, the lid of which was removed remotely after

five rounds of alternating stimuli. Females were free to move about the arena for up to five

minutes, and a choice was scored once the female reached a 10cm "choice area" in front of

a speaker. For each trial, the non-preferred stimulus was broadcast at a constant amplitude

of 85 dB SPL, while the preferred stimulus was started at 85dB, and then lowered in 3 dB

steps in successive trials until the preference reversal occurred. Stimulus amplitudes were

verified using a Lutron SL-4001 sound-level meter (fast RMS with 'C' weighting) prior to each test, and the source of the stimuli was reversed at random during successive trials to guard against side bias. Females were rested between their individual trials for no more than 15 minutes. Previous research with *H. cinerea* has determined that there are no carry-over effects between trials (Gerhardt 1981a).

Experiment 1: Intraspecific Experience

Experience Phase: Male green tree frogs produce short calls ranging from 100-200ms in length, which are repeated roughly 80 times per minute. Spectrally, the calls contain two frequency bands, one in the low frequency range (0.65 - 1.5 kHz), and the other in the high frequency range (2.5 - 4 kHz) (Gerhardt 1974; Gerhardt et al. 1987). While calls vary between individuals (Gerhardt et al. 1987), as well as geographically (Asquith et al. 1988), females express preferences based on call duration, relative amplitude, call rate, and frequency (Gerhardt 1974, 1980; Gerhardt et al. 1987; Höbel 2010). The strongest of these factors is call frequency (Gerhardt et al. 1987; Höbel 2010). Preference functions for call frequency show geographic variation in *H. cinerea*, ranging from open ended functions favoring low frequency calls to closed functions favoring intermediate frequencies (Höbel and Gerhardt 2003). Before starting the social plasticity experiment we therefore tested 11 females to obtain information on the local preference function shape.

To generate the preference function, we used a series of six two-choice trials that presented a standard 900Hz call (grand species average) against three lower and three higher alternatives (in 100 Hz steps), thus covering a range of 600 to 1200 Hz (this slightly extends the species range). During each trial, the chosen stimulus was awarded a score of

"1", the rejected stimulus a score of "0". Because each female was confronted with each of the alternative stimuli only once, but heard the standard stimulus in each of her six trials, we calculated the final score of the standard stimulus as [(sum of standard scores across all trials) / 6]. We then used the R Package "PFunc" (Kilmer et al, subm.), to generate a population level preference function.

Because the preference function for call frequency in our study population is open-ended favoring low frequency calls (Figure 1.1), we generated a low-frequency call (dominant frequency of 0.8 kHz) to serve as a preferred (attractive) stimulus, and a higher-frequency call (dominant frequency of 1.1 kHz to serve as a less preferred (unattractive) stimulus. As is tradition for *H. cinerea* call stimuli (Gerhardt 1976), we generated the synthetic stimuli to have one frequency component in the low–frequency range, and two frequency component in the high–frequency range (i.e., 0.8+2.4+2.7 kHz, and 1.1+3.6+3.9 kHz).

Temporal parameters were equalized between the two stimuli: call duration was 160ms, call rise and fall times were set to 25ms and 50ms, respectively. Using the generated unattractive and attractive stimuli, we manipulated the composition of the social environment using four treatments: Attractive calls only, Unattractive calls only, a Mixed treatment featuring both attractive and unattractive calls at the same ratio, and a Silence treatment. During experience treatments, stimuli were repeated on average every 400ms.

Testing Phase: During the testing phase, females were given a choice between the unattractive (high-frequency) call broadcast at a constant 85dB SPL, and the attractive (low frequency) call was attenuated each round until the female ceased to approach the attractive call.

Experiment 2: Interspecific Experience

Experience Phase: Over the eastern portion of their range, green tree frogs are sympatric with the barking tree frog, *Hyla gratiosa* (Conant and Collins 1998). Green and barking tree frogs are sister species (Wiens et al. 2010), and their calls have similar acoustic structure: temporal call traits (duration, rise time) are similar, the frequency range partially overlaps, but the dominant frequency and harmonic structure of the calls are different (Oldham and Gerhardt 1975). Both species are genetically compatible (Mecham 1960), and documented hybridization in syntopic populations (Gerhardt et al. 1980; Höbel and Gerhardt 2003) suggests interspecific social interaction. Hybrids between the two species are not sterile, and gene introgression via backcrosses and continued hybridization has been observed in syntopic populations. However, F1 hybrids are at significant reproductive disadvantage with parental species (Höbel & Gerhardt 2003) and hybrid populations do suffer break down without the continued presence of both parental species (Schefler et al. 1986). Female green tree frogs prefer the calls of conspecifics over those of barking tree frogs, but will approach barking tree frog calls if those are presented alone, or if amplitude differences strongly disfavor the conspecific call (Oldham and Gerhardt 1975; Gerhardt 1981; Höbel and Gerhardt 2003).

To manipulate social experience involving the presence of conspecific and heterospecific, we generated a preferred (conspecific) stimulus with the spectral components of an average green tree frog call (0.9 + 2.7 + 3.0 kHz) (Gerhardt 1987; Höbel and Gerhardt 2003), and a non-preferred (heterospecific) stimulus with the spectral components of an average barking tree frog (0.5 + 1.5 + 2.0 kHz) (Oldham and Gerhardt

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1975; Gerhardt 1981; Höbel & Gerhardt 2003). Note that conspecific calls not only are higher in frequency overall, but also differ in their frequency pattern (i.e., 300 vs. 500 Hz periodicity). Temporal parameters were equalized between the two stimuli: call duration was 150 ms, call rise and fall times were set to 25ms and 50ms, respectively. Using these synthetic calls as representative of green and barking tree frogs, we manipulated the composition of the social environment using four treatments: Conspecific calls only, Heterospecific calls only, a Mixed treatment featuring the conspecific and heterospecific call at equal ratio, and Silence treatment featuring no calls. During experience treatments, stimuli were repeated on average every 900ms. This lower repetition rate accounts for the longer call periods typical for barking treefrogs.

Testing Phase: During the testing phase, females were given a choice between the heterospecific call broadcast at a constant 85dB SPL, and a conspecific call that was attenuated each round until females ceased to approach the conspecific call.

Statistical Analysis

We performed a population-based analysis in which we expressed female choosiness data as the percentage of females still approaching the preferred stimulus at each attenuation level. For each experiment we used a mixed model implemented in JMP Pro (Version 12.1.0 SAS Institute Inc., 2015). We entered the percentage of females approaching the preferred stimulus as the dependent variable, and terms for treatment, attenuation and the treatment x attenuation interaction as test variables. When significant differences were detected between groups, we used a Post Hoc Tukey Kramer test of Honest Significant Difference to determine which groups differed significantly from one another.

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RESULTS

Experiment 1: Intraspecific Experience

Sixty-three females completed the playback trials (15-18 individuals in each treatment). As amplitude differences between the attractive (preferred) and the unattractive (non-preferred) call increased, fewer females continued to approach the preferred call (Figure 1.2.; Table 1.1: Effect of Attenuation). The experience treatment significantly affected the amplitude difference at which females began to approach the unattractive call; females were more choosy after experience with an environment that contained attractive males (attractive and mixed) compared to females that had experienced an environment that did not contain attractive males (unattractive and silent; Figure 1.2; Table 1.1).

Experiment 2: Interspecific Experience

Eighty-three females completed the playback trials (18-22 females per treatment). Again, as amplitude differences between the conspecific (preferred) and the heterospecific (non-preferred) stimulus increased, fewer females continued to approach the preferred call (Figure 1.3.; Table 1.2: Effect of Attenuation). Females were similarly choosy after experience with a pure conspecific, pure heterospecific or silent treatment. By contrast, experience with a mixed chorus made females less choosy (Figure 1.3; Table 1.2: Effect of Treatment x Attenuation).

DISCUSSION

We explored social plasticity in choosiness in female green tree frogs in response to variation in the chorus environment. We found that treatments manipulating female experience of chorus composition influence choosiness, and this was the case when the treatments involved variation of the intra- as well as inter-specific social environment.

In the intraspecific context, we had predicted that females that experience attractive calls would be more choosy than females that experience unattractive ones, or no calls at all. Our prediction was supported, as we observed higher choosiness during the attractive and mixed treatments (i.e., when attractive males were perceived to be present), and lower choosiness in the unattractive and silence treatments (i.e., when attractive males appeared to be absent). This type of social plasticity in choosiness seems to have an adaptive function, as it allows females to adjust the effort expended in mate choice based on the availability of mate types. By being choosy if preferred mates are available, females can focus investment in securing a preferred mate to occasions where it is worthwhile to do so. But continued searching for preferred mates when they are rare wastes energy and increases risk of predation (Magnhagen 1991, Grafe 1997), and by being too choosy females may also miss out on mating opportunities altogether. By accepting a wider range of males via decreased choosiness, females can reduce these mating costs.

The pattern of social plasticity in choosiness observed in green treefrogs is consistent with a hypothesis initially proposed for the evolution of social plasticity in mate preferences – the mating assurance hypothesis (Fowler-Finn and Rodríguez 2012a,b). The function of this plasticity in mate preference selectivity is to secure mating with preferred types when those are available, yet ensure to not forgo a reproductive opportunity

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altogether when the preferred types are rare or absent. This pattern of social plasticity in

mate preferences has been observed in a range of taxa, including insects, spiders, and fish

(Rebar, D., Zuk, M. & Bailey, N.W. 2011, Bailey and Zuk 2009 Kozak and Boughman 2009,

Wagner 2001 Hebets and Vink 2007). Finding that social plasticity in choosiness is

consistent with the mating assurance hypothesis suggests that selection favoring plasticity

that balances choice of optimal mates with guarding against forgoing reproduction may be

widespread in nature.

In the interspecific context, we had predicted that social plasticity in response to

between-species variation would mirror the response to within-species variation. We

predicted that females that experienced conspecific calls would be more choosy than

females that experience heterospecific ones, or no calls at all, because it would assure that

females invest more in mate sampling if it is worthwhile (i.e., if there are conspecific males

present). This was not the case. Not only did females decrease choosiness during one of the

treatments that presented conspecifics (the mixed treatment), the lack of a corresponding

change after experience of the conspecific-only treatment also indicates inconsistent social

plasticity.

Behavioral responses vis-a-vis heterospecifics are frequently couched in terms of

mismating avoidance, based on an assumed maladaptive outcome of interspecific mating

interactions (Gröning & Hochkirch 2008). Surprisingly, experiments that manipulate the

level of experience with heterospecifics can have a variety of outcomes. Sometimes,

experience with heterospecifics results in increased discrimination (Kozak and Boughman

2009, Kozak et al 2013, Svensson et al 2010, Magurran and Ramnarine 2004). This pattern

indeed supports a hypothesis of mismating avoidance, in which plasticity allows for

stronger or less tolerant preferences in the presence of heterospecifics in order to decrease the risk of hybridization (Rodriguez et al. 2013). However, in other instances, experience with heterospecifics results in decreased discrimination, leading to higher acceptance of heterospecifics when conspecifics are rare or absent (Fowler-Finn Rodriguez 2012b). There are also examples where experience with heterospecifics leads to a preference for the heterospecific as a result of mate copying or imprinting (Verzijden & ten Cate, 2007 Kozak et al., 2011). The results of our study also highlight the diversity of outcomes resulting from experience with heterospecifics, as experience with a mix of con- and heterospecifics reduced choosiness for the conspecific call, while experience with heterospecific alone did not induce plasticity in choosiness.

Reproductive interactions between green and barking tree frogs do occur throughout the sympatric portion of their range, and can span the gamut from long-term hybridizing populations in disturbed habitats (Schefler et al 1986) to accentuated differences in calls and preferences in sympatry compared to allopatry (i.e., reproductive character displacement; Höbel and Gerhardt 2003). This suggests that the cost of hybridization can vary, and that the pattern of social plasticity in choosiness observed in our study, although likely increasing hybridization should both mate types become present at that site, may not always be maladaptive. For example, in the context of an ongoing range expansion of green tree frogs into the north central part of the range of barking tree frogs, whereby previously allopatric populations are currently coming into secondary contact (Lodato et al. 2014), we speculate that social plasticity in choosiness would lead to increased hybridization. Here, plasticity may facilitate the initial stages of range expansion because hybridization is preferable to forgoing reproduction altogether. The above

considerations also suggest that the pattern of social plasticity in choosiness reported here is not representative of the behavior of the species as a whole. Because the green tree frog population at our study site has no history of contact with barking tree frogs, social plasticity vis-a-vis heterospecifics has likely not been under selection for an adaptive function. The pattern of plasticity we observed may thus represent the original plasticity unmodified by selection. Sampling sites with differing levels of sympatry, evolutionary history, or environmental quality may help elucidate the sources of selection acting on the evolution of social plasticity in the interspecific context.

We have demonstrated that female green tree frogs show social plasticity in choosiness across a range of social contexts, but with notably different outcomes for the adaptiveness of the resulting patterns in mate choice decisions. We suggest that the likelihood with which females encounter a particular social environment may play a central role in the evolution of the observed patterns in social plasticity. The variable, but persistent, presence of conspecifics may have allowed for the evolution of adaptive social plasticity in the conspecific context. By contrast, the absence of barking tree frogs from the study area never allowed for selection on plasticity in response to the presence of heterospecifics, and any observed pattern represents unselected plasticity. Exploring the forces that shapes plasticity across social environments and populations may help explain variation in mate choice decisions and their evolutionary consequences.
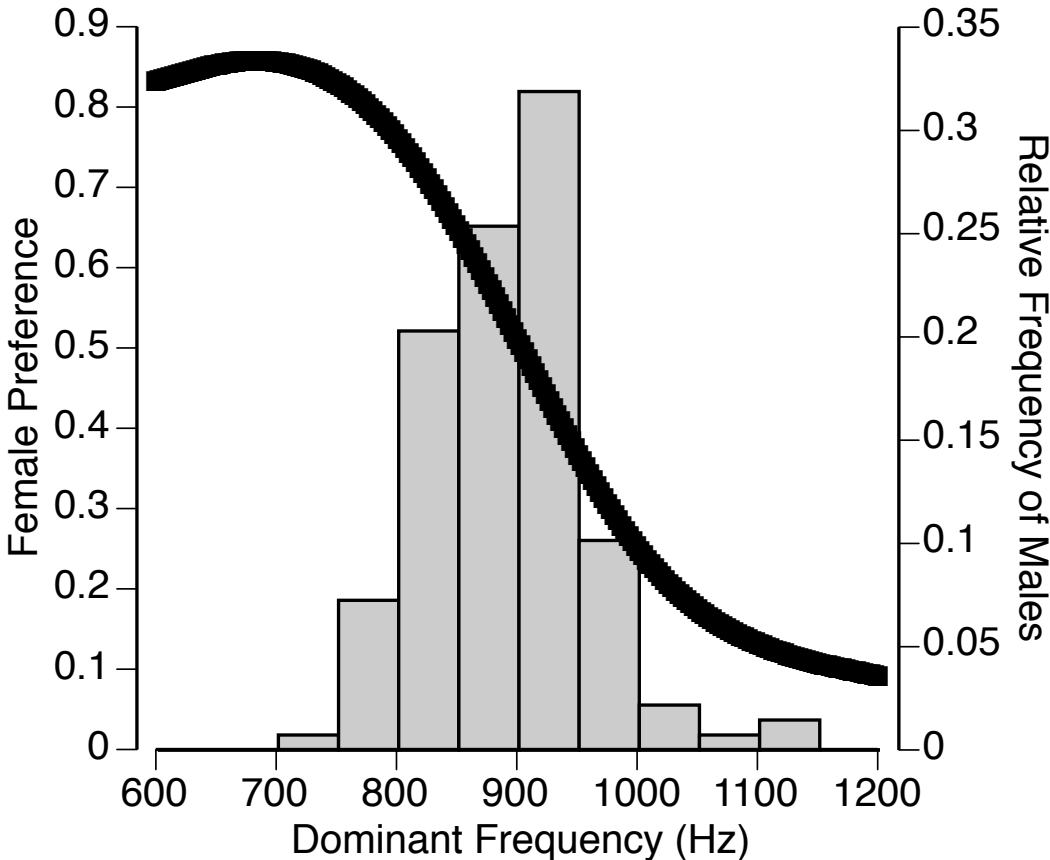
Figure 1.1 Population level preference function for dominant frequency peak of females in Jasper, TX (n = 11), overlaid with the distribution of the dominant frequency peak of male advertisement calls in the population (n-138). Bin size is 50Hz.
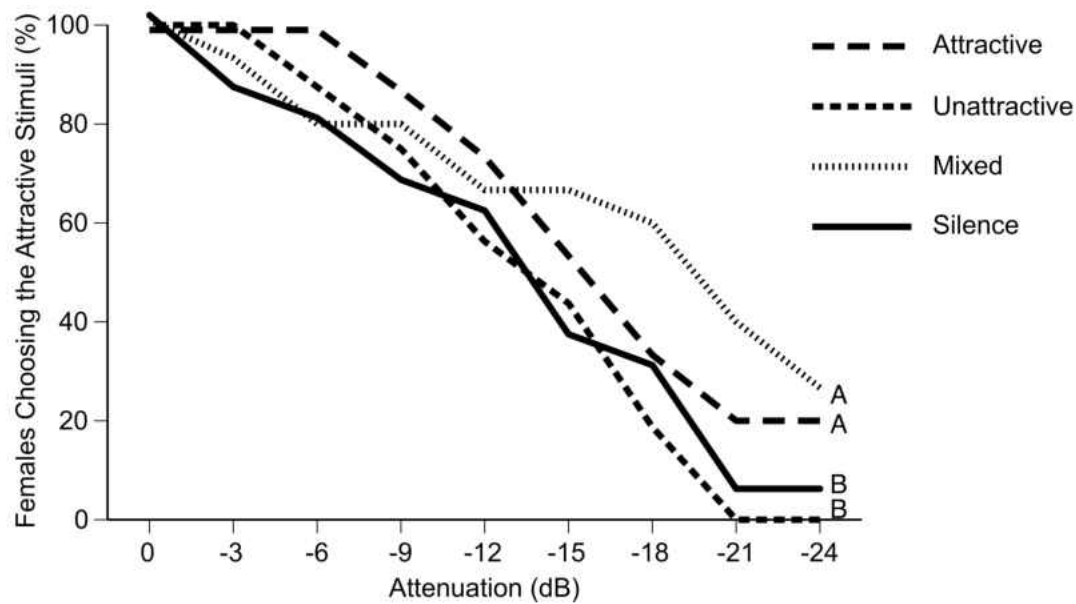
Figure 1.2 Choosiness during the intraspecific trials, expressed as the percentage of females still choosing the preferred (attractive) stimulus as it is gradually attenuated. A post hoc Tukey Kramer HSD test determined that females in the attractive and mixed treatments were significantly more choosy than females in the unattractive and silence treatments. Lines not connected by the same letter are significantly different.
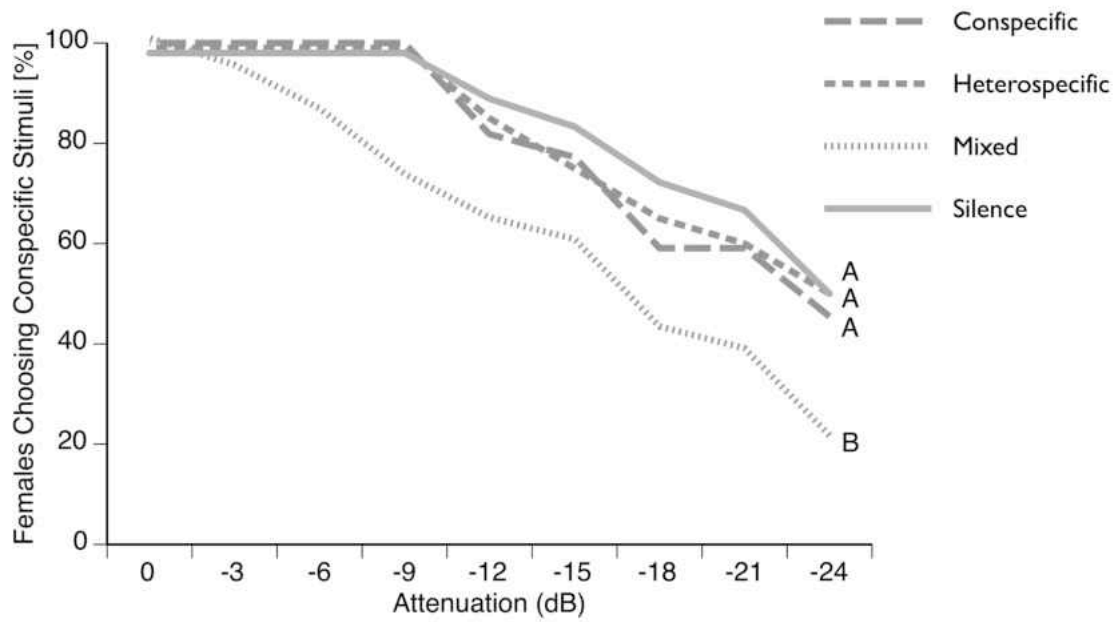
Figure 1.3 Choosiness of females in the interspecific trials, expressed as the percentage of females still choosing the preferred (conspecific) stimulus as it is gradually attenuated. A post hoc Tukey Kramer HSD test determined that females in the mixed treatment were significant less choosy than females in the conspecific, heterospecific, or silence treatments. Lines not connected by the same letter are significantly different.

Table 1.1: Mixed model results for intraspecific social plasticity in choosiness

Effect	df	*F*	*p*
Treatment	3,58	5.31	<0.001
Attenuation	1,61	570.11	0.005
Treatment X Attenuation	3,58	5.89	0.003

Table 1.2: Mixed model results for interspecific social plasticity in choosiness

Effect	df	*F*	*p*
Treatment	3,79	17.68	<0.001
Attenuation	1,81	358.97	<0.001
Treatment X Attenuation	3,79	3.6	0.0254

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On The Architecture of Mate Choice Decisions: Preference Functions and Choosiness are

Distinct Traits

ABSTRACT

Mate choice is an important cause of sexual selection; it can drive the evolution of

extravagant ornaments and displays, and promote speciation through the reproductive

isolation generated by rapid divergence of sexual traits. Understanding mate choice

requires knowledge of the traits involved in generating mating preferences, and how those

traits may interact with each other. It has been proposed that mate choice decisions are the

outcome of two components that vary independently: the *preference function* (the ranking

of the attractiveness of prospective mates) and *choosiness* (the effort invested in mate

assessment). Here we examine individual variation in female preference functions and

choosiness for the dominant frequency of the male advertisement call in Green Treefrogs

(*Hyla cinerea*) to test two predictions of this hypothesis. We show that measures of

preference function traits and measures of choosiness do not correlate with each other. We

also show that both components respond differently to different causes of variation:

preference function traits varied mostly with preference function shape, and were related

to body size only for individuals with closed preferences. By contrast, choosiness did not

vary between individuals with closed or open preferences, and was related to body size

only in individuals with open preferences.

INTRODUCTION

Over 20 years ago, Jennions and Petrie (1997) discussed the causes and consequences of

variation in mate choice in terms of the underlying architecture of mate preferences. Here

they identified two components: *mate preference functions* (the ranking of prospective mates) and *choosiness* (the effort invested in mate assessment)*.* To avoid confusing the term "mate preference" with the term "preference function", we suggest referring to the broad topic for analysis as the mate choice decision; we also suggest viewing choosiness as the effort expended to or willingness to invest in acquiring the preferred mate type, rather than solely mate assessment effort, to distinguish it from mate-searching strategies (Janetos 1980; Parker 1983; Dombrowsky & Perrin 1994). With this modified framework, the hypothesis proposed by Jennions and Petrie (1997) can be stated as follows: variation in mate choice decisions arises from the interaction of preference functions and choosiness.

The first component, *the preference function,* has long been recognized for its usefulness in studying sexual selection, and characteristics of preference functions have been used to generate hypotheses about selection on male traits (Ritchie 1996, Wagner 1998, Shaw and Herlihy 2000; Andersson and Simmons 2006; Rodriguez et al. 2006, 2013a). Initially, studies focused on the overall shape of the preference function — open-ended and favoring extreme male trait values or closed and favoring intermediate male trait values — as well as on the relationship between the peak (the most preferred trait value) and the male trait distribution. Recent refinements in the conceptualization and analysis of preference functions have further dissected them to reveal additional *preference function traits*, which allow for a more fine scale and quantitative analysis (Fowler-Finn and Rodriguez 2012a,b; Rodriguez et al 2006, 2013b, Kilmer et al. 2017). These preference function traits can provide insight into a female's acceptance of trait values that deviate from her peak (tolerance), her variation in response across trait values (strength), and a female's average response across all trait values (responsiveness) (Figure. 2.1).

The second component, *choosiness*, can also affect the strength and direction of selection on male traits as female preference functions are best expressed when females are choosiest. For example, two females could have the same preference function, but vary in their choosiness: the choosier female invests more effort in mate selection, and thus is more likely to obtain her most preferred male. By contrast, a female that is not very choosy likely mates with a male whose traits do not strongly reflect her preference function. Variation in this second component appears to have received less attention in the sexual selection literature than preference functions (but see Lindstrom and Lehtonen 2013; Judge et al. 2014; Neelon and Höbel 2017, Kuczynski et al 2017).

Jennions and Petrie (1997) proposed that preference functions and choosiness are distinct traits that can evolve independently. Whether this is the case or not is a key question in the theory of sexual selection and speciation (Mead & Arnold 2004; Kuijper et al. 2012; Kopp et al. 2018). Theoretical studies vary in whether they view preference functions and choosiness as independent or related in various ways (see discussion in Kopp et al. 2018). Some researchers measure willingness to acquire preferred types separately from preference functions and use various terms for such measures, including choosiness (Chenoweth & Blows 2006; Gerhardt et al. 2000; Höbel & Gerhardt 2003; Lindstrom and Lehtonen 2013; Judge et al. 2014; Kuczynski et al 2017; Neelon and Höbel 2017), while others use the term choosiness to refer to an intrinsic aspect of preference functions (Gray & Cade, 1999; McPeek & Gavrilets, 2006; Bailey, 2008; Edward, 2015; Reinhold & Schielzeth, 2015; Rosenthal 2017).

Whether variation in preference functions is independent or related to variation in the willingness to secure preferred types is an empirical question, regardless of how one

refs to those components. The answer is of fundamental importance, however: either the

mate types that are most attractive evolve independently of how much effort is exerted in

obtaining them, or they do not; this determines whether or not preferences and choosiness

should be conceptualized as corresponding to different loci. (cf. Kopp et al. 2018).

We tested the hypothesis that preference functions and choosiness are independent

traits (Jennions and Petrie 1997). To our knowledge, the relationship between preference

functions and choosiness as conceptualized by them has not been assessed empirically.

This hypothesis makes two predictions. First, variation in preference functions should not

be correlated with variation in choosiness. Second, preference functions and choosiness

should respond differently to different causes of variation.

To test these predictions we took advantage of the well-studied acoustic

communication system of Green Treefrogs, *Hyla cinerea* (Anura: Hylidae). Green Treefrogs

are a common anuran species that has been the focus of intense research on

neurophysiological, behavioral, and evolutionary aspects of mate choice and sexual

selection (Gerhardt 1974, 1981; Gerhardt et al. 1987; Höbel and Gerhardt 2003, Höbel

2010). We used playback experiments to describe individual variation in preference

functions and choosiness in relation to each other and to two factors that commonly

influence them: body size and social experience (Hebets and Sullivan-Beckers 2010;

Fowler-Finn & Rodríguez 2012a,b; Rodriguez et al. 2013c; Wong et al. 2011; Neelon and

Höbel 2017, Kuczynski et al 2017).

METHODS AND MATERIALS

Study Species and Sampling

Our focal population of *H. cinerea* inhabits the western part of the species' range, at the East Texas Conservation Center, in Jasper, TX. We performed all trials during May-July of 2013. During the breeding season (April-July), males congregate at ponds and swampy areas where they produce advertisement calls to attract females. The calls are short, ranging from 100-200ms in duration, with a repetition rate of 80 calls/min. Calls contain two spectral bands, in the low frequency range (0.68 - 1.2 kHz), and the high frequency range (2.3 - 3.7 kHz) (unpubl.). Females show preferences for a number of call traits (i.e., duration, relative amplitude, and repetition rate), but the strongest preference is for call frequency (Gerhardt 1974, 1981; Gerhardt et al. 1987; Höbel 2010). There is geographic variation in preference functions and in choosiness for call frequency (Höbel and Gerhardt 2003). There is also evidence of socially mediated plasticity in choosiness (Neelon and Höbel 2017).

We collected females in the late evening at the beginning of chorus formation from amplexed pairs to ensure sexual receptivity. We released all frogs at the site of capture after the conclusion of the night's trials. Experimental procedures were approved by the Animal Care and Use Committee of the University of Wisconsin-Milwaukee (IACUC 07-08#38).

Testing Preference Functions and Choosiness

We used synthetic playback stimuli varying in their spectral features (see below) and fixed at the mean values of our focal population for duration (160ms), rise time (25ms), and fall time (50ms). Females readily respond to these synthetic calls via phonotaxis. We used a custom-written DOS program (courtesy of J.J. Schwartz) (available upon request) to generate the playbacks.

We tested females in an outdoor arena set up at our study site, but away from active choruses and at a time when males generally had ceased calling (between 0100 -0500 hrs). The arena consisted of a plywood floor (2×1 m) surrounded by a wood frame 50cm in height, screened with a visually opaque but acoustically transparent black cloth. Just outside of the cloth, two loud speakers (JBL Control 1X) were placed opposite one another along the central long axis of the arena, so that females could hear the broadcast stimuli, but not see the speakers. Stimuli were broadcast from a PC laptop using Audacity® software (Audacity Team 2013). Stimulus amplitudes were verified using a Lutron SL-4001 sound-level meter (fast RMS with 'C' weighting) prior to each test.

During each playback trial, females were placed in an acoustically transparent 10 cm-wide by 5cm-tall wire cage in the center of the arena, the lid of which was removed remotely after five rounds of alternating stimuli. Females were able to climb out of the cage and free to move about the arena for up to five minutes, and a choice was scored once the female reached a 10cm "choice area" in front of a speaker. Females were rested 5-15 min between consecutive trials.

To examine the relationship between preference functions and choosiness, we obtained data on both for each female. We switched each night the order of playback trials conducted to describe preference functions or choosiness, to guard against sequence/time biases. We detected no such biases: neither preference function traits nor choosiness differed between females whether either was obtained first or second that night (*t*-tests: *p* ≥ 0.08).

Preference functions

Preference functions are representations of how attractiveness varies with ornament trait values (i.e., Ritchie 1996; Rodríguez et al. 2006). Because mate preferences are expressed in terms of the ornaments encountered by an individual, they are function-valued traits best represented as curves (Meyer and Kirkpatrick 2005; Stinchcombe et al. 2012; Rodríguez et al. 2013b; Kilmer et al. 2017). Variation in these functions can be analyzed in terms of their overall shape: open preference functions increasingly favor extreme trait values, and closed preference functions favor intermediate trait values and discriminate against extremes). Within these shape categories, the curves can be further characterized by *preference function traits*. These include *peak* (a females most preferred trait value), *tolerance* (how willing a female is to accept trait values that deviate from her peak), *strength* (the overall variation in female response across trait values) and *responsiveness* (a female's average response across all trait values (see Figure. 2.1) (Fowler-Finn & Rodríguez 2012a,b; Kilmer et al. 2017). Note that preference shape and the different preference function traits may in principle vary independently; e.g., peak may vary to a similar extent

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within closed and open preferences (Figure. 2.1A). We therefore tested for differences in preference function traits according to the overall shape of the preferences.

To describe female mate preference functions for call frequency we conducted a series of six two-choice trials, using synthetic calls generated as described above. Trials presented a "standard" call with a dominant frequency of 900Hz (grand species average) against alternatives that were either lower (600, 700, 800 Hz) or higher (1000, 1100, 1200Hz) in dominant frequency. The frequency range covered by these trials (600 - 1200 Hz) slightly exceeds the natural range of the species, as is recommended to capture the full shape of the preference function within biological relevance (Rodríguez et al. 2013; Kilmer et al. 2017). We randomized stimulus sequences for each female and periodically switched the speaker presenting the standard call (every seven trials) so that each female was tested with both speaker configurations.

To obtain stimulus-specific response values from our two-choice data structure where females chose between two alternatives, we scored the trials as follows: The chosen alternative was awarded a score of "1", the rejected alternative a score of "0". Because each female was presented with each of the alternative stimuli only once, but presented with the standard stimulus in all of her trials, we calculated the final score of the standard stimulus as [(sum of standard scores across all trials) / 6]. Scores for each of the six alternatives could thus be either 0 or 1, and scores for the standard could range from 0-1 (Figure. 2.2A).

We then used a function-valued approach to describe mate preference functions on the basis of these scores. We used the program PFunc (Kilmer et al. 2017) to fit non-parametric regressions for each individual tested (Figure. 2.2A). This method makes no assumption about the shape of the functions, other than that they should have some level of

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smoothness (e.g., it does not pre-specify a linear or quadratic shape, but allows each function to be determined by the responses of the individual females). We then used the program to calculate the above preference function traits for each individual female. This method has been used extensively to measure individual, genetic and plasticity-related variation in preference functions (Reichert & Höbel 2015; Rodriguez et al. 2013; Underhill & Höbel 2017). Finally, we obtained a population function and population function traits.

Choosiness

Our assay of choosiness is based on the natural behavior of Green Treefrog females: if a female has a choice between an attractive male that is farther away and a less attractive male that is nearer, she will often choose the closer, less attractive male. We manipulated apparent distance to the playback stimuli by changing their relative amplitude — as per the inverse square law of sound attenuation, amplitude decreases by 6 dB for each doubling of distance to the sound source (Speaks 1997). This offers a simple but effective assay of the distance a female is willing to go in securing the mate she prefers (Gerhardt et al. 2000, Höbel and Gerhardt 2003; Neelon and Höbel 2017, Kuczynski et al. 2017).

We assessed choosiness with two-choice playback trials presenting an unattractive call (1100 Hz) played at constant amplitude against an attractive call (800 Hz) that is attenuated. For each trial, the unattractive alternative was broadcast at a constant amplitude of 85 dB SPL. To start a trial, we presented the attractive alternative at 73 dB SPL, and then adjusted its amplitude up or down in steps of 6 dB and then 3 dB until we identified the lowest amplitude at which she still approached the quieter attractive stimulus rather than the louder unattractive stimulus. Arriving at a choosiness measure for

each female thus required 3-4 trials. The lowest amplitude required to arrive at these choosiness scores (61 dB SPL) is well within the auditory threshold (derived from single auditory fiber recordings) of *H. cinerea* (30dB SPL; Ehret and Capranica 1980) and the phonotaxis threshold of a related species of similar size (43 dB SPL*; Hyla versicolor;* Beckers and Schul 2004). As with the preference function trials, we switched speaker locations periodically.

Causes of variation

One of the predictions of the hypothesis that preference functions and choosiness are independent traits is that they will be influenced differently by variables such as social experience and body size.

Social Experience Treatments

We manipulated the social experience of females using playbacks simulating variation in chorus composition. Females were randomly assigned to one of four experience treatments: (i) attractive stimulus only (800Hz playback), (ii) unattractive stimulus only (1100Hz playback); (iii) a 1:1 mixture of the unattractive and attractive stimuli; or (iv) a silent treatment (no playback). The body size of females entering each experience treatment did not differ significantly ($F_{3,57}$ = 1.46, *p* = 0.24).

We created the treatment playbacks with Audacity® by pasting the synthetic stimuli into longer sound files that we played to females. The temporal pattern of the calls in the sound file mimicked natural male chorusing behavior, with a mean inter-call interval

of 400 ms that ranged from the equivalent of one male in isolation to several interacting

males (Höbel and Gerhardt 2007).

We broadcast the playbacks from a MP3 player (RCA TH2002RDR) through an

iHome rechargeable mini speaker (iHM60) adjusted to a sound pressure level of 73dB,

which is representative of natural chorus noise (Velez et al 2012). These were placed next

to mesh screen cages (Exo Terra Explorarium; 45x60cm) containing 2-5 females. The

playback apparatus and cages were placed into quiet areas of the study site (i.e., away from

active frog choruses). Females entered the experience treatments immediately after

capture (21:00-22:00 hrs), and remained exposed to the playbacks for 3 hours.

Immediately after the conclusion of their playback treatment females were tested for their

preference functions and their choosiness.

Variation in body size

We took advantage of the natural size variation of reproductively active females in our

study population (range: 34.5-56.8mm, n = 63) to test whether the relationship between

body size and preference functions was different from the relationship between body size

and choosiness. We measured body size as snout-vent-length (SVL), which is measured

from the tip of the nose to the end of the vent.

Statistical Analysis

We tested the hypothesis that preference functions and choosiness are independent traits

in two ways. First, we looked at trait correlations. If preference functions and choosiness

are independent, the function traits (peak, tolerance, strength, responsiveness) may be

correlated with each other, but they should not be correlated with choosiness. We ran this analysis separately for open and closed preferences because preference function traits differed according to the shape of the preferences (see below).

Second, we examined how social experience and body size relate to preference function traits and choosiness. Here the prediction is that if they are indeed independent, then any changes in preference function traits associated with variation in body size or social experience should be uncorrelated with any changes choosiness. We tested this using standard least square regressions that had either function traits (peak, tolerance, strength, responsiveness) or choosiness as the dependent variable. The models had the following explanatory terms: preference function shape, female body size, and social experience treatment. Initially, we included all two-way and three-way interactions. The three-way interactions were never significant ($p \geq 0.26$), and we removed them to increase statistical power.

These analyses involved multiple tests with traits that were correlated (peak, tolerance, strength, and responsiveness; see below). Although this may increase the risk of spurious significance (Rice 1988), corrections for multiple testing compromise statistical power (Moran 2003; Nakagawa 2004). To deal with this potential problem, we followed a table-wide criterion for analyzing significance tests (Moran 2003). This criterion revealed that our tests do not suffer from this risk: significant differences were widespread and diverse across the traits described (see below).

All statistical analyses were performed using JMP Pro v. 11.1.0 (SAS Institute Inc., 2015).

RESULTS

Variation in Preference Functions and Choosiness

Hyla cinerea females varied in the shape of their preference functions. Out of 62 individuals

for which we completed the full battery of trials, 53% had open functions, 42% had closed

functions, and 5% had flat functions (Figure. 2.2B). We removed the three females with flat

preference functions from further analysis because those females respond similarly to

every tested call trait value and thus did not allow us to extract meaningful preference

function traits. Body size differed only slightly between females with open and closed

preferences (mean ± SD SVL = 47.8 ± 5.1 vs 45.6 ± 4.2 mm, respectively), and this 4%

difference was barely detectable (*t*-test: t = 1.74, df = 55.6, *p* = 0.09). Function shape was

also not influenced by social experience (χ^2_3=0.19, *p* = 0.98). [nota bene: removal of the

females with flat preferences did not bias our subsequent analysis: females with flat or

curved (either open or closed) functions did not differ significantly in body size (t-test: *t* = -

1.70, df = 2.18, *p* = 0.22) or choosiness (*t* = -2.14, df = 2.27, *p* = 0.15), and flat functions were

not more common after certain experience treatments (χ^2_3 =3.63, *p* = 0.30)].

Individual females varied widely in their preference function characteristics (both

shape as well as function traits; Figures. 2.2, 2.3, 2.4). All preference function traits (peak,

tolerance, strength and responsiveness) differed significantly between functions of open

and closed shape (Figure. 2.3.)

Individual females also varied in choosiness, and the range of choosiness scores

across all females spanned the gamut from 0 to 24 dB attenuation difference (mean ± SD:

13.3 ± 6.9 dB). This range of choosiness is remarkable, especially when translated from dB

difference to the distance the choosy females are willing to walk to reach their preferred

mate. Using the inverse square law of sound attenuation, a very choosy female with a 24dB score would walk 16 m further than a non-choosy female with a 0dB score (only willing to walk to the more attractive call if it is equidistant to the unattractive one).

Correlation between preference functions and choosiness

Preference function traits (peak, tolerance, strength and responsiveness) were highly correlated with each other, but were almost never correlated with choosiness (Table 2.1).

Preference function shape played an important role for determining correlations between different preference function traits, as well as between function traits and choosiness. Only two out of six trait correlations (Tolerance - Strength, and Peak - Responsiveness) were similar in sign and magnitude between closed and open preference functions, while four trait correlations were different in sign and/or magnitude (Table 2.1). Further, only in open preference functions was a preference function trait (Strength) significantly correlated with choosiness — but here our criterion for analysis discounts this difference as potentially spurious (Moran 2003), and in any case the correlation was not strong (Table 2.1).

Relationship of body size and social experience with preference functions and choosiness

The variables we assessed related differently to preference function traits and choosiness (Table 2.2, Figure. 2.4). Briefly, preference function traits differed between individuals with open and closed preferences, and were related to body size only for individuals with closed preferences (with different traits showing different relationships with body size). By

contrast, choosiness did not vary between individuals with closed or open preferences, and was related to body size only in individuals with open preferences. Social experience never had an effect. We detail these results below.

Preference Peak significantly varied with preference shape (Table 2.2). Females with open preferences had significantly lower peaks (least square mean ± SE: 623 ± 9 Hz) than females with closed preferences (803 ± 11 Hz). Body size, and the size × function shape interaction also had an effect: large females, especially those with closed functions, had lower peaks (Figure. 2.4 A,B).

Preference Tolerance significantly varied with preference shape (Table 2.2). Females with closed preferences had significantly narrower tolerance (least square mean ± SE: 211 ± 16 Hz) than females with open preferences (256 ± 14 Hz) (Figure. 2.4 C,D).

Preference Strength significantly varied with preference shape (Table 2.2). Females with closed preferences had significantly higher preference strength values (1.85 ± 0.20) than females with open preferences (0.73 ± 0.16). Body size, and the size × function shape interaction also had an effect: small females with closed functions had higher preference strength values (Figure. 2.4 E,F).

Preference Responsiveness significantly varied with preference shape (Table 2.2). Females with open preferences were significantly more responsive (0.42 ± 0.02) than females with

closed preferences (0.34 ± 0.02). Body size also had an effect, with larger females being more responsive (Figure. 2.4 G,H).

Choosiness was significantly affected by the size × preference shape interaction (Table 2.2). Larger females with open preferences were choosier (Figure. 2.4 I,J).

DISCUSSION

Our results support the hypothesis that mate preference functions and choosiness are independent traits (Jennions and Petrie 1997). In our population of *H. cinerea*, preference function traits and choosiness were predominantly un-correlated, and variation in preference function traits and choosiness was associated with different variables. This finding has several implications for our understanding of sexual selection via mate choice and its consequences for speciation. First, the absence of a phenotypic correlation between the preference function traits and choosiness suggests that the genetic correlation too may be absent or weak. If so, preference functions and choosiness may evolve independently, each being tweaked or optimized by natural and sexual selection, potentially without major trade-offs. Additionally, it also supports the intuition that preferences and choosiness should be modeled as being influenced by different loci (cf. Kopp et al. 2018), and that choosiness should not be equated with aspects of the preference function such as what we term tolerance, strength, and responsiveness (see discussion in Kilmer et al. 2017). More studies in other species and groups are necessary to determine how widespread our findings may be.

A second interesting point is that variation in preference functions and choosiness, whether between-individuals, populations or species, may interact in ways that bring about various evolutionary consequences. For instance, choosiness may determine the strength of selection due to mate choice, while the tolerance of the preference function may determine the amount of variation permitted around the peak of the preference (Figure. 2.1). If so, choosiness may determine the speed at which equilibrium is attained, and preference tolerance may in turn determine the variation sustained at equilibrium. The interplay between preference functions and choosiness may generate considerable variation in the resulting patterns of assortative mating and signal-preference linkage disequilibrium, as well as in the consequences for the maintenance of genetic variation and the promotion of divergence. A related question: which is a stronger determinant of the strength of sexual selection due to mate choice: choosiness or the preference function traits that describe its curvature around the peak.

Also of particular interest is our finding of considerable variation in all the components of mate choice: overall preference shape, the different preference function traits, and choosiness. The population-level preference function had a closed shape with a peak beyond the population mean for the call trait (Figure. 2.2C). This would suggest moderately strong directional selection on male call frequency. However, the population contained near equal numbers of females with open and closed preferences. Their relative contributions to selection on signals will vary according to their choosiness, as per the above rationale. However, their contributions will also vary in an additional sense: The peak of open preferences was further away from the male mean than the peak of the closed preferences (Figure. 2.2C). Consequently, females with open preferences favor more

extreme male signals than females with closed preferences — although their greater tolerance, lower strength and higher responsiveness suggest they would exert overall weaker selection. These patterns of variation suggest how female preferences may actually be involved in the maintenance of variation in signals. While relatively understudied, there is evidence that such within-population variation may be common, at least in terms of individual differences in peak preference, preference tolerance, and whether females have a preference or not (Wagner et al. 1995; Jennions and Petrie 1997; Hedrick and Weber 1998; Murphy & Gerhardt 2000).

Both preference functions and choosiness varied continuously, suggesting inputs from many loci. This seems to be common in nature, at least for preference functions (Rodriguez et al. 2013b), as well as the ability of preference functions and choosiness to be modified by variables that span the gamut from the age, condition, or reproductive stage of the choosing individual to the social environment they experienced during certain stages of their life (Rosenqvist and Houde 1997; Hunt et al. 2005; Byers et al. 2006; Uetz and Norton 2007; Hebets and Sullivan-Beckers 2010; Fowler-Finn and Rodríguez 2012a,b; Rodriguez et al. 2013c; DuVal and Kapoor 2015; Neelon and Höbel 2017, Kuczynski et al. 2017). We find that if a given factor changes the preference function, it will likely not change choosiness, or change it in a different way. For example, body size affected both function traits and choosiness, but for function traits it was the closed functions that were mostly affected, while for choosiness it was the open functions. One outcome of this independent influence is a higher likelihood that the plastic response by each of these components may also be shaped independently by selection.

In conclusion, we find that preference functions and choosiness are distinct traits that may interact in various ways to generate mate choice decisions. A full understanding of how mate choice contributes to sexual selection and speciation will require the joint study of variation in both of these components to establish whether this pattern is widespread in nature. It will also require assessing how these components interact with other determinants of mate choice decisions, such as the mate sampling rules that are followed by individuals varying in preferences and choosiness (Jennions and Petrie 1997; Wagner 1998; Uy et al. 2001; Cotton et al. 2006).

Figures and Tables
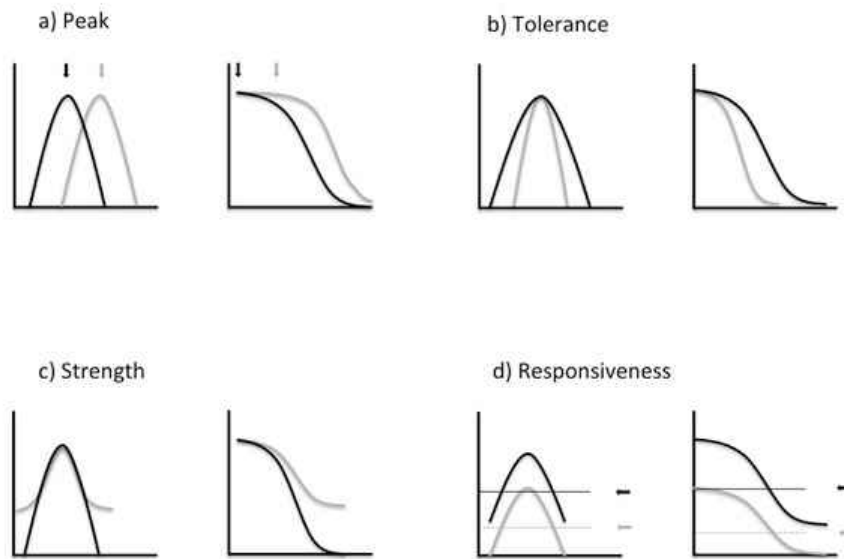
Figure 2.1 Description of preference function shapes and associated preference function traits.

Preference function shape can be closed, and favor intermediate male trait values (left panels) or open-ended and favoring extreme male trait (right panels). Preference function traits obtained for our study were: (A) peak, the most preferred trait value, (B) tolerance, a measure of willingness to accept trait values that deviate from the peak, (C) strength, the variation in response across trait values, and (D) responsiveness, the average response across all trait values. Note that overall preference shape and preference function traits may in principle vary independently, such that and variation in one function trait does not predetermine variation in the other traits (black vs. gray curves), and a given function trait may vary to a similar extent within closed and open preferences (arrows). For example, (A) peak may vary to a similar extent within closed and open preferences (depending in the case of open preferences of whether and where they plateau), or (B) tolerance may vary in functions that have the same peak, responsiveness and strength.
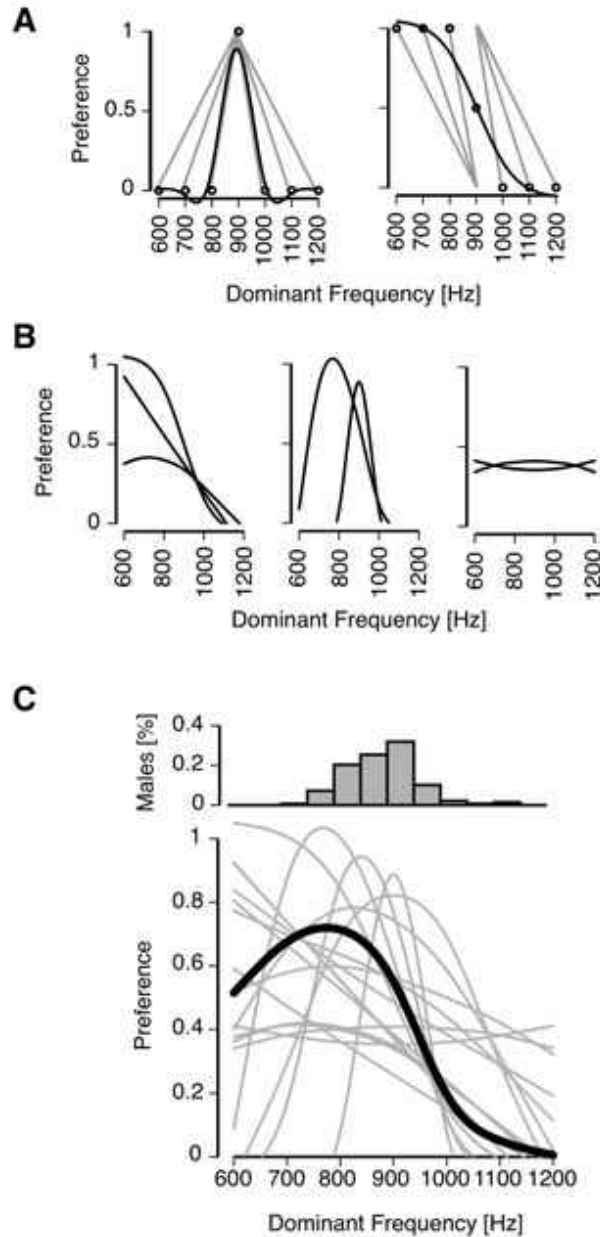
Figure 2.2 Variation in preference functions for the low frequency peak of the male advertisement call. (A) Preference functions were obtained by conducting two-choice trials (gray lines indicate stimulus exemplars tested in each trial), which were converted into preference scores (symbols), onto which non-parametric splines were fitted (black line). Shown are an example of a closed (left) and an open (right) preference function. (B). Functions could be grouped into three general shapes: open (53% of females; left), closed (42% of females; center), or flat (5% of females; right). (C) Comparison between the distribution of the dominant frequency peak of male advertisement calls in the population (n = 138; bin size is 50 Hz) with the preference functions of individual females (gray lines), and for comparison, the composite function for the population (black line).
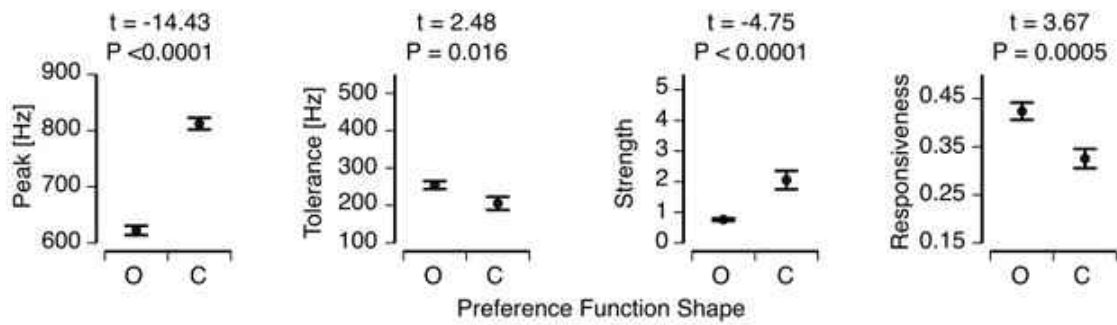
Figure 2.3 Differences in preference functions traits between open and closed preference functions. Size of y-axis indicates range of individual variation in preference trait values. Shown are mean ± SE, and the results of t-tests comparing function shapes.
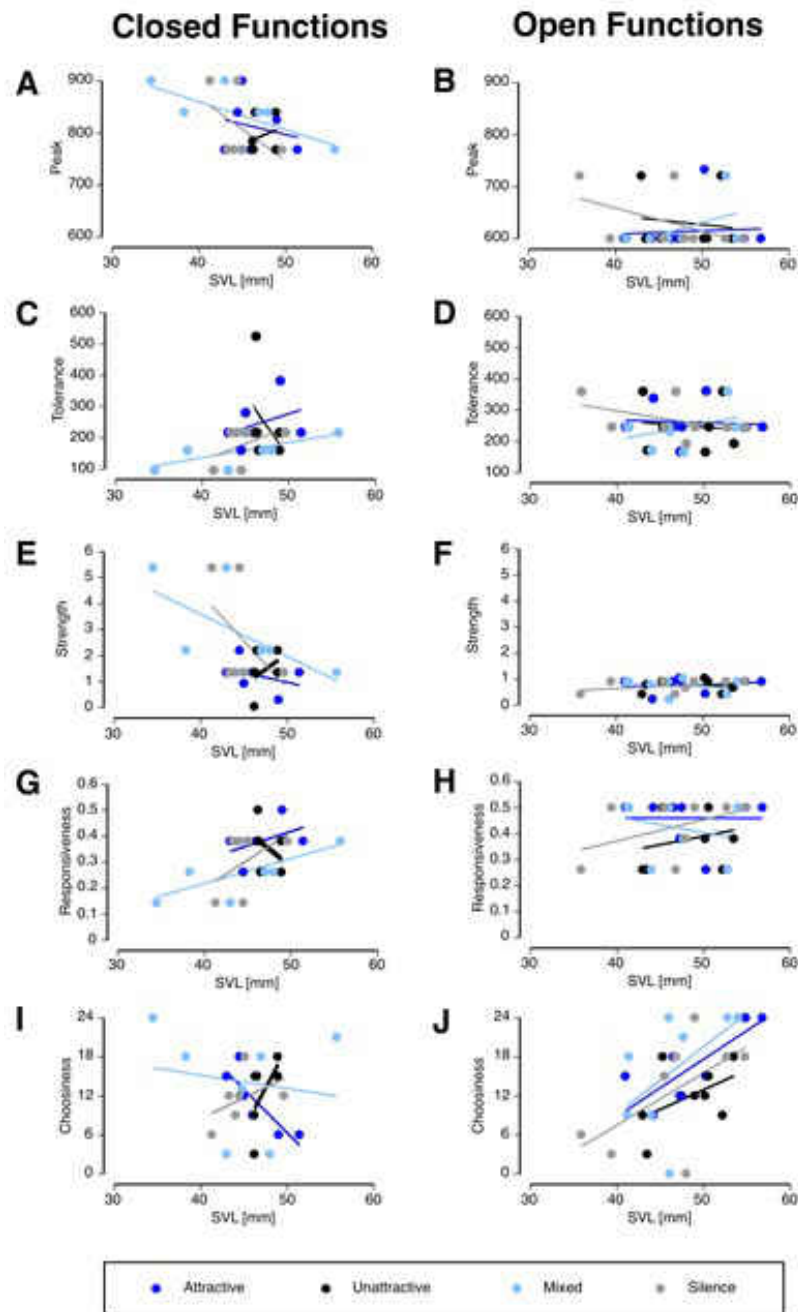
Figure 2.4 Influence of social experience, body size, and function shape on female mating preferences. Shown are preference function traits (A-H) and choosiness (I,J) separate for females with closed (left column) and open (right column) preference functions. Preference functions traits were mainly affected by preference functions shape and body size, while choosiness was only affected by the size × function shape interaction. Details are provided in the main text.

Table 2.1: Correlations between peak preference, tolerance, strength, responsiveness and choosiness. The strongest correlations are those between the four preference functions traits. Correlations calculated separately for closed and open preference functions; significant correlations are set in bold.

Shape	Trait	Tolerance	Strength	Responsiveness	Choosiness
Closed	Peak	**-0.44**	**0.76**	**-0.77**	-0.04
	Tolerance		**-0.76**	**0.86**	-0.17
	Strength			**-0.94**	0.01
	Responsiveness				-0.10
Open	Peak	**0.83**	**-0.68**	**-0.76**	-0.07
	Tolerance		**-0.70**	-0.33	0.05
	Strength			**0.51**	**0.38**
	Responsiveness				0.28

Table 2.2: Results of a standard least squares model examining the effects of social experience treatment, body size and preference function shape on preference function traits and choosiness (see Fig. 2.4).

Peak	Factor	DF	*F*	*p*
	Treatment	3,45	0.18	0.907
	Female size	**1,45**	**4.46**	**0.040**
	Function shape	**1,45**	**173.43**	**<0.0001**
	Treatment × Female size	3,45	1.20	0.322
	Treatment × Function shape	3,45	0.86	0.466
	Female size × Function shape	**1,45**	**4.18**	**0.047**
Tolerance	Factor	DF	*F*	*p*
	Treatment	3,45	1.30	0.287
	Female size	1,45	0.01	0.906
	Function shape	**1,45**	**4.58**	**0.038**
	Treatment × Female size	3,45	0.45	0.716
	Treatment × Function shape	3,45	0.92	0.441
	Female size × Function shape	1,45	0.30	0.588
Strength	Factor	DF	*F*	*p*
	Treatment	3,45	1.71	0.179
	Female size	**1,45**	**4.23**	**0.046**
	Function shape	**1,45**	**20.12**	**<0.0001**
	Treatment × Female size	3,45	0.13	0.944
	Treatment × Function shape	3,45	1.47	0.237
	Female size × Function shape	**1,45**	**5.79**	**0.020**
Responsiveness	Factor	DF	*F*	*p*
	Treatment	3,45	1.29	0.289
	Female size	**1,45**	**4.47**	**0.040**
	Function shape	**1,45**	**8.59**	**0.005**
	Treatment × Female size	3,45	0.77	0.517
	Treatment × Function shape	3,45	0.88	0.461
	Female size × Function shape	1,45	2.68	0.109
Choosiness	Factor	DF	*F*	*p*
	Treatment	3,45	0.91	0.442
	Female size	1,45	1.56	0.218
	Function shape	1,45	0.95	0.334
	Treatment × Female size	3,45	0.11	0.957
	Treatment × Function shape	3,45	0.59	0.662
	Female size × Function shape	**1,45**	**5.91**	**0.019**

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Staying ahead of the game –plasticity in chorusing behavior allows males to remain attractive in different social environments

ABSTRACT

Dense breeding aggregations are highly dynamic environments that place extreme pressure on advertising males to remain competitive in the face of rivals. The assemblage of males at breeding sites may vary substantially in their relative attractiveness to one another, and in the case of anurans and insects, may also include heterospecific males that provide interference to a signaling male. There is evidence that in response to this challenge, males use plasticity in signal features or signal presentation to maximize their relative attractiveness in the face of competitors. However, the costs associated with this plasticity may select for the evolution of mechanisms such as selective attention, by which males exhibit the greatest plasticity in calling behavior in response to the most relevant competitors, while disregarding less relevant rivals. We used two playback experiments - one simulating the intraspecific environment and the other simulating the interspecific environment - to test the hypothesis that males use social plasticity in signal features, signal presentation, or both, to remain attractive in response to rivals. Further, we examined whether this plasticity is mediated by selective attention to the most relevant competitors. We find that males do exhibit plasticity in temporal features of the call (signal feature), while relying mostly on signal timing (signal presentation) to remain attractive in response to rivals. We further show that this plasticity is most pronounced in response to the most relevant competitors (attractive/conspecific males). We discuss the relationship of these male behaviors to female preferences, as well as the consequences for the evolution of group displaying behaviors.

INTRODUCTION

In the cacophony and chaos of dense breeding aggregations, there can be intense pressure

for individuals to stand out from the crowd. Consider the choruses of acoustically

advertising insects and anurans, where males produce signals to attract females, and

females select males based on a number of signal traits. The simultaneous advertisement of

many males creates highly dynamic social environments, in which the relative audibility

and attractiveness of a male may change rapidly as other males join or leave the chorus.

Moreover, mixed species choruses are common in nature, and often include species whose

signals overlap partially in frequency composition or temporal structure (Gerhardt 1982,

Höbel and Gerhardt 2003, Reichert and Gerhardt 2014). Although a fixed signaling strategy

would at times be sufficient to attract a female, selection should favor males with plastic

signaling behavior that can adjust their relative attractiveness in response to variations in

the social environment.

Social plasticity in displays, i.e., the ability to change display traits in response to the

displays of a rival, falls into two broad categories. First, a male may exhibit plasticity in his

display features (Wells and Taigen 1986, Lopez et al 1988, Schwartz 1993, Bee and Perrill

1996). In anurans for example, this can involve changes in the temporal properties of the

call, such as lengthening the call duration (Wells and Taigen 1986, Bee and Perrill 1996), or

adjusting spectral properties of the call, such as lowering the dominant frequency (Lopez et

al. 1988, Wagner 1989, Bee et al 2000). Second, a male may show plasticity in *signal*

presentation in response to rivals (Buck 1938, Grafe 1999, Greenfield 2015), often

specifically in a way that reduces signal interference (Schwartz 1993, Klump and Gerhardt

1992, Greenfield 2015) . Such changes in signal presentation can involve very different time frames, ranging from initiating choruses at different hours of the night to avoid interference from heterospecifics (Greenfield 1993) to males trading individual signals or even individual signal elements (Grafe 2003, Martinez & Gerhardt 2008). One benefit of precise timing of individual signals is to maximize its salience and ensure that it can be perceived clearly by mate searching females. In addition, females of a variety of taxa also show preferences for the temporal order of signals, generally preferring the leading of two signals in close succession (Minckley and Greenfield 1995; Greenfield 1997; Snedden 1998; Greenfield and Snedden 2003, but see Grafe 1999). In these cases, females tend to discriminate against signals that are perceived in overlapped, lagging position, as well as those that follow the leading signal too closely (within the "forbidden interval", Greenfield 1997). Not surprisingly, males of these species try to avoid placing their signals in such unattractive positions (Greenfield and Snedden 2003, Greenfield et al. 1997, Snedden and Greenfield 1998, Greenfield and Rand 2000, Höbel 2011)

Although altering one's call features and/or presentation in response to changes in the social environment seems beneficial, it may have associated costs (Wells and Taigen 1986, Marin-Cruz & Greenfield 2016). For example, it would be impractical for a male to attend and adjust to every single rival within the breeding aggregation simultaneously, since this would greatly reduce his amount of signaling (Greenfield and Rand 2001). Rather, since females are the selective agents, males may maximize the efficiency of social plasticity in signaling behavior by attending only to those rivals that females would simultaneously assess. In chorusing species, those closest competitors are generally the males nearest neighbors (Grafe 1996, Greenfield and Snedden 2003, Reichert and Gerhardt 2013). There

is indeed evidence that males do exhibit such *selective attention* in signal timing behavior, by adjusting to at most three of their loudest (i.e., nearest) neighbors (Greenfield & Rand 2000). Whether males are able to distinguish relative attractiveness of their nearest neighbors, and then preferentially attend to the more relevant (attractive / conspecific) rival while disregarding the less relevant one (unattractive / heterospecific) is currently unknown.

We conducted playback experiments that presented focal male Green Treefrogs (*Hyla cinerea*) with simulated social environments that varied in the composition and attractiveness of potential rivals. We conducted two experiments: one featuring only conspecific playbacks with simulated rivals that differed in attractiveness, and a second one featuring conspecific as well as heterospecific playbacks (i.e., mixed-species chorus), where we consider the conspecific to be the more relevant (i.e., attractive) stimulus.

We use these trials to test the hypothesis that males use social plasticity in signal features, signal presentation, or both, to remain attractive in different social environments. To our knowledge this is the first study to assess both types of signaling plasticity simultaneously, allowing for a direct comparison of which type is most prevalent. The hypothesis that males use *call plasticity* to remain competitive makes the following predictions: (i) calls should be different when males call alone (control) compared to when they interact with rivals (playback treatments); (ii) males should change their call features in ways that increase their attractiveness to females. In the case of Green Treefrogs, this includes lowering the call frequency, increasing the call duration, and decreasing the call period (Gerhardt 1974, 1981; Gerhardt et al. 1987; Höbel 2010); (iii) stronger competition (playback containing attractive/conspecific rival) should result in stronger response. The

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hypothesis that males use *call timing* to remain competitive makes the predictions that (i) males should overlap fewer calls than expected by chance, (ii) stronger competition (attractive/conspecific calls) should result in stronger overlap avoidance, and (iii) stronger competition (attractive/conspecific call) should result in more pronounced call inhibition (i.e., longer call delays and longer post-stimulus inhibition). In addition, we also test a prediction of the selective attention hypothesis, namely that when two signals that differ in relevance are presented together, males should preferentially attend to the more relevant one (here, attractive / conspecific).

METHODS AND MATERIALS

Study Species and Study Site

Green tree frogs, *Hyla cinerea*, (Anura: Hylidae), are a common anuran species found throughout the southeastern United States (Conant and Collins 1998). Each night from April through July, males congregate in choruses at ponds and swampy areas, where they produce advertisement calls to attract females. The calls are short, ranging from 100-200ms in duration, with a repetition rate of 50-80 times per minute. Calls contain two spectral bands, in the low frequency range (0.68 - 1.2 kHz), and the high frequency range (2.3 - 3.7 kHz) (unpubl. data from recordings of n = 548 males across the species range). Males often aggregate in large choruses, and since their range overlaps with several other hylids, they may share breeding ponds with several other species, including members of the same genus (*H. versicolor. H. chrysoscielis, H. gratiosa, H.squirella*; pers. obs; Gerhardt 1974; Gordon et al 2017). Depending on habitat type (*H. cinerea* is relatively unspecific in the type of breeding habitat they accept, in contrast to several other species that prefer

smaller, fishless habitats) and time in breeding season (breeding seasons of different species show only partial overlap), choruses can be single species or they can be multi-species. (pers obs., Höbel and Gerhardt 2003, Gordon et al 2017). Female *H. cinerea* show preferences for a number of male call features (i.e., call frequency, duration, relative amplitude, and repetition rate), as well as male call presentation (signal timing traits) (Gerhardt 1974, 1981; Gerhardt et al. 1987; Höbel 2007, 2010).

Our focal population of *H. cinerea* inhabits the western part of the species' range, at the East Texas Conservation Center, in Jasper, TX, where we performed all trials during May-July of 2012 and 2013. All experimental procedures were approved by the Animal Care and Use Committee of the University of Wisconsin-Milwaukee (IACUC 07-08#38)

Stimulus Generation

Synthetic calls of *H. cinerea* traditionally consist of one frequency component in the low–frequency range, and two frequency components in the high–frequency range. We generated synthetic advertisement calls using a custom-written DOS program courtesy of J.J. Schwartz (available upon request), and then used Audacity® software (Audacity Team 2013) to create longer stimulus playbacks that we presented to males at the pond. Males readily interact with call playbacks, and will engage in signal timing with synthetic stimuli (Höbel and Gerhardt 2007).

Intraspecific Experiment

To test the hypothesis that males use social plasticity of call behaviors to maintain attractiveness in the face of intraspecific variation in the social environment, we generated stimuli to represent variation in relative attractiveness of potential conspecific

competitors. Although female *H. cinerea* show preferences for a number of call traits, the strongest preference is for call frequency (Gerhardt 1974, 1981; Gerhardt et al. 1987; Höbel 2010) Accordingly, we simulated variation in the social environment using playbacks varying in call frequency.

Preference functions for call frequency show geographic and local variation in *H. cinerea* female preferences, ranging from open-ended preference functions favoring low frequency calls to closed preference functions favoring intermediate frequencies (Höbel and Gerhardt 2003, Neelon and Höbel in Prep). At our study population, the population level preference function is open-ended favoring low frequency calls. Accordingly, we created an attractive, low-frequency call stimulus (0.8+2.4+2.7 kHz), and an unattractive, high-frequency call stimulus (1.1+3.6+3.9 kHz). Temporal characteristics of the call stimuli were equalized to 160ms call duration (with a call rise time of 25ms and a fall time of 50ms). These specific synthetic calls have previously been shown to be attractive and unattractive to females of this population (Neelon and Höbel 2017).

We used information on male call-timing behavior (see Höbel and Gerhardt 2007) to simulate the presence of several interacting males. This was accomplished by pasting the synthetic call stimulus at randomly varied inter-call intervals. Average inter-call interval was 400ms (equivalent to two males calling antiphonally), but the range of intervals used was representative of one male calling alone to several males interacting with each other).

To generate the treatment simulating an attractive social environment, the stimulus playback only contained the attractive 800Hz call; the unattractive treatment only contained the unattractive 1100Hz call (with identical temporal presentation of calls). To generate the mixed treatment, we alternated the attractive stimulus with the unattractive

stimulus, resulting in a 1:1 ratio of call types (again, identical temporal presentation of calls).

Interspecific Experiment

To test the hypothesis that males use social plasticity of call behaviors to maintain attractiveness in a social environment that also contains heterospecifics (i.e., interspecific variation in the social environment), we confronted calling males with calls of conspecifics as well as those of Barking Treefrogs (*Hyla gratiosa*). We chose Barking Treefrogs as the heterospecific stimulus for two reasons. First, their calls have very similar acoustic structure; temporal call traits are very similar, and although the dominant frequency is lower and the harmonic structure of the calls are different, there is nevertheless substantial overlap in the frequency range between the calls of both species (Oldham and Gerhardt 1975). Second, both species are broadly sympatric across the eastern portion of the Green Treefrog range (Conant and Collins 1998).

We modeled the call stimulus representing the conspecific male after an average *H. cinerea* call (0.9 + 2.7 + 3.0 kHz) (grand species average across the specie's range; Gerhardt 1987; Höbel and Gerhardt 2003), and the call stimulus representing the heterospecific male after an average *H. gratiosa* call (0.5 + 1.5 + 2.0 kHz) (Oldham and Gerhardt 1975; Gerhardt 1981; Höbel & Gerhardt 2003). Note that *H. cinerea* calls not only are higher in frequency overall, but also differ in their harmonic structure (i.e., 300 vs. 500 Hz periodicity). Temporal parameters were equalized between the two stimuli: call duration was 150 ms and call rise and fall times were set to 25ms and 50ms, respectively.

To generate the treatment simulating a conspecific social environment, the stimulus playback only contained *H. cinerea* calls; the heterospecific treatment only contained *H. gratiosa* calls (with identical temporal presentation of calls). To generate the mixed treatment, we alternated the *H. cinerea* stimulus with the *H. gratiosa* stimulus, resulting in a 1:1 ratio of call types (again, identical temporal presentation of calls). During these playback treatments, stimuli were repeated on average every 900 ms. This lower repetition rate accounts for the longer call periods typical for *H. gratiosa*.

Male Testing and Recording

Male playback trials were conducted during the time active choruses formed (2100-2400hrs). We chose focal males that were actively calling but had no calling neighbor within 5 m, to ensure that our playback would be perceived as the most pertinent rival. We used an MP3 player (Sandisk Sansa Clip+ SDMX18R-002GK-A57) connected to a speaker (Optimus XTS 40) to broadcast the treatment stimuli to the focal males. The speaker was placed one meter away and at equal height of the calling male (depending on the male's location we placed the speaker on the ground or on a tripod). We used a Marantz PMD670 digital recorder and a Sennheiser K3-ME88 microphone to record the focal males (sampling rate of 44.1 kHz, saved as PCM files).

We conducted the conspecific experiment in 2012, and the heterospecific experiment in 2013. At the beginning of each trial, the spontaneous calling of the focal male was recorded for two minutes. Then we started to broadcast the playback treatments. Playback treatments lasted 5 minutes each, followed by 2 minutes of silence before the next treatment was broadcast. Treatments were presented in random order. A full playback trial

consisted of three playback treatments (attractive/unattractive/mixed, or

conspecific/hetrospecific/mixed, respectively). At the end of each playback trial we

measured ambient temperature.

Call Analysis

To analyze the recording from the playback treatments, we used the program Avisoft

SAS-LabPro to measure call features, and the program Raven Pro 1.5 (Cornell Lab of

Ornithology Bioacoustics Program) to measure relative call timing of the focal males.

Call features - We measured two spectral and three temporal properties of the calls: (i)

first frequency peak, (ii) second frequency peak, (iii) call duration (length of call), (iv) call

rise time (time from the start of the call to its maximum amplitude), and (v) call period

(time between the beginning of one call and the beginning of the following call; Figure 3.1

a). We measured 5 calls (selected for optimal signal-to-noise ratio) from each treatment as

well as the pre-playback recording. Each male thus contributed four sets of five calls each

to the analysis. We use this data to test the hypothesis that males use call feature plasticity

to maintain attractiveness in different social environments.

Call timing – We measured call delays (time from onset of stimulus to onset of focal

male call), and then used this data to obtain measures of call overlap avoidance and

estimates of two temporal properties of the male call timing behavior. (Figure 3.1b,c). For

the playback treatments that only contained one type of call stimulus (i.e., the attractive

and unattractive treatment in the intraspecific experiment, and the conspecific and

heterospecific treatment in the interspecific experiments), we measured the delays of 50

calls. For the playback treatments that contained two types of call stimuli (i.e., the mixed

treatments in the intraspecific and the interspecific experiments), we measured the delays of 50 calls per stimulus (for example, during the mixed treatment in the intraspecific experiment we measured call delays after 50 attractive and 50 unattractive stimuli). Thus, this analysis focused on equalizing sample size across stimulus types, not across treatments.

From the measured call delay values we computed three call timing measures (see also Höbel 2015): (i) To examine call overlap avoidance, we computed the proportion of overlapping lagging calls. Since we knew the duration of the playback stimuli (160 ms in the intraspecific experiment, and 150 ms in the interspecific experiment), every call delay measure below this value corresponded to a call given in overlapped, lagging position. We focused on overlapped lagging calls because these are strongly discriminated against by females (Höbel and Gerhardt 2007; Höbel 2010).

To examine temporal parameters of call timing behavior, we transformed call delay measures (time from stimulus onset to call onset) to call latency measures (time from stimulus offset to call onset). We did this by subtracting the duration of the respective stimuli (150 ms or 160 ms, depending on experiment) from the initial call delay measures. We focused on call latency because male *H. cinerea* use the offset of interfering stimuli to time their own calls (Höbel and Gerhardt 2007). We did not measure call latency directly because the shape (i.e., amplitude envelope) of the *H. cinerea/H. gratiosa* call (and their synthetic equivalent), with its steep onset but relatively shallow offset, makes it much harder to determine the end of the stimulus than the beginning. Since we knew the duration of each presented stimulus, we were able to take advantage of the steep stimulus onset for more precise measurements.

From this latency data, we determined (ii) the duration of post-stimulus suppression (i.e., the timing of the earliest call the male gave after the end of a stimulus, indicating the end of the time period during which calling is no longer suppressed) and (iii) the modal call latency (i.e., the timing at which the male placed most of its calls). (Figure 3.1b,c) We chose the mode rather than the mean to describe call timing behavior because it more realistically reflects male call timing behavior (see Höbel and Gerhardt 2007; Höbel 2015). We consider suppression and modal latency to be different traits, because the timing of the first call after a stimulus might be due to a physiological, stimulus-induced suppression response, whereas the timing of most calls could be due to a male choosing a particular timing relationship between the stimulus and his call.

We use this data to test the hypothesis that males use call timing to maintain attractiveness in different social environments. Note that although individual males contributed 50 call delay measures per stimulus type, for the final analysis we obtained only one call overlap measure, one post-stimulus suppression value and one modal latency value per stimulus type per focal male.

Statistical Analysis

We ran analyses separately for each experiment (intraspecific and interspecific social environment, respectively). All statistical tests were computed using JMP Pro v. 13.1.0 (SAS Institute Inc., 2015)

Analysis of call feature plasticity – Since calls are multivariate traits, and the constituting call properties may be correlated with each other, we conducted the analysis in two steps.

First, we ran a principal component analysis including all five call traits. For each experiment (intraspecific and interspecific social environment) the PCA returned two principal components with eigenvalues larger than 1, and in each case the spectral (first and second frequency peak) and temporal (call duration, rise time and period) call properties loaded together into one of the principal components (see below).

Separate for each experiment we used mixed models (implementing REML) with post-hoc student's *t* tests to examine whether males changed their calls in response to different social environments. We first entered the principal components as response variables, treatment and temperature as test variables, and male ID as a random term. We entered temperature in the model because it is known to affect call properties in frogs (Gerhardt and Huber 2002), and we entered male ID to account for each male contributing 5 call measurements per treatment. For testing our hypothesis, we are only interested in the effect of treatment.

This initial analysis revealed that in both experiments social environment did affect the principal component related to temporal call parameters (see below). We therefore dissected the effect of social environment on temporal call features further by running additional mixed models that had call duration and call period, respectively, as response variables (and test variables as described above). We focused on these two parameters because they are known to influence female choice in *H. cinerea* (Höbel 2010), while it is currently unknown whether females have preferences for call rise time.

Analysis of call timing plasticity – We used mixed models (implementing REML) with post-hoc student's *t* tests to examine whether males adjusted their call timing in response to different social environments. We entered % call overlap (arcsine square root

transformed before analysis), post-stimulus suppression and modal call latency, respectively, as the response variables, and treatment, stimulus type (nested within treatment) and temperature as test variables. Since call timing is in effect a temporal call trait, we entered temperature in the model to account for any temperature effects. Note also that because frogs time their calls in response to immediate signal interference, the important comparison for testing our hypothesis is not the treatment term, but the stimulus type term.

RESULTS

We recorded n=19 males during the Interspecific trials, and n=9 males during the intraspecific trials. Final sample size was reduced in some tests because males ceased calling during some treatments.

Intraspecific Experiment

Call Plasticity

The principle component analysis returned two principal components with eigenvalues larger than 1. PC1 had an eigenvalue of 2.19, and loaded principally with the spectral parameters (first frequency: 0.61, second frequency: 0.60). PC2 had an eigenvalue of 1.31, and loaded mainly with the three temporal call parameters (Call duration: 0.52; Call rise time: 0.67; Call Period: 0.49). Together the two first PC's accounted for 69.9% of the variation (Figure 3.2a).

The mixed model identified treatment as a significant determinant of variation in PC2, the principal component that correlated with temporal call parameters (Table 3.1, Figure.

3.2). Further analysis focusing on individual temporal traits revealed significant effects of treatment on duration and period: Compared to the control, during which males called alone, call duration increased across all playback treatments (Table 3.2, Figure 3.2b). Call period did not differ from the control during the unattractive treatment, but was significantly longer during the mixed and especially during the attractive treatment (Table 3.2, Figure 3.2c).

Call Timing

All three measures of call timing behavior were affect by the presented stimulus type (Table 3.3, Figure. 3.3). Males overlapped fewer calls with the attractive stimulus than the unattractive one (Figure. 3.3a), and both post-stimulus suppression (Figure. 3.3b) as well as modal call latency (Figure. 3.3c) were longer after attractive compared to unattractive stimuli. Moreover, in the mixed treatment that presented both attractive and unattractive stimuli, males responded more strongly to the attractive stimulus (Figure. 3.3a-c), although this only reached statistical significance for post-stimulus suppression. The last result suggests that males show selective attention when confronted with two signal types that differ in behavioral importance.

Interspecific Experiment

Call Plasticity

The principle component analysis returned two principal components with eigenvalues larger than 1. PC1 had an eigenvalue of 1.60, and loaded principally with the three

temporal call parameters (Call duration: 0.65; Call rise time : 0.48; Call Period:0.52). PC2 had an eigenvalue of 1.22, and loaded mainly the spectral parameters (first frequency: 0.63, second frequency: 0.55). Together the two PC's accounted for 56.4% of the variation (Figure. 3.4a).

The mixed model identified treatment as a significant determinant of variation in the principal component that correlated with temporal call parameters (PC1) (Table 3.4, Figure. 3.4). Further analysis focusing on individual temporal traits revealed significant effects of treatment on call duration and call period: Call duration significantly increased during the conspecific and mixed treatments relative to the control, with call duration showing the greatest increase during the mixed treatment (Figure. 3.4b, Table 3.5). Call period increased relative to the control across all treatments (Figure. 3.4c, Table 3.5).

Call Timing

Only call overlap was significantly affected by the presented stimulus type (Table 3.6, Figure. 3.5). Males overlapped fewer calls with the conspecific stimulus than with the heterospecific one (Figure. 3.5a), and this was particularly clear during the mixed treatment.

DISCUSSION

Male Green Treefrogs showed social plasticity in call features as well as call placement, but to remain attractive in different social environments, they relied mostly on changes in call timing behavior. Moreover, males were very attuned to the attractiveness of the simulated rivals: social plasticity was more pronounced during the conspecific than during the mixed-species experiment, and when simultaneously confronted with rivals that differed in attractiveness (mixed treatments in each experiment), males showed selective attention by attending primarily to the more relevant (attractive/conspecific) stimulus.

Female Green Treefrogs show preferences for a number of call traits, including longer call duration and shorter call period. The strongest preferences in this species, however, is for call frequency (Gerhardt et al 1987). In this population, males with lower-frequency calls are more attractive (Neelon & Höbel 2017), and lowering call frequency would have been an adaptive strategy when confronted with rivals. Some frog species are indeed capable of changing call frequency (Lopez et al. 1988, Bee et al 2000, Wagner 1989), but in most species spectral call parameters seem to be largely determined by the morphology of the call production mechanism (McClelland et al 1996), and accordingly males of Green Treefrogs may not have been capable to modulate their spectral call traits.

Males increased call duration during playback trials (compared to the controls), which should increase their attractiveness to females. However, they simultaneously increased call period, which should make them less attractive to females. At first glance, prolonging call periods is a counterintuitive response, yet is the logical outcome arising from the strong plasticity in signal timing we observed during the playback trials: avoiding call overlap requires delaying a males own call until after the offset of the interfering stimulus,

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which by default will result in longer call periods. Particularly when interacting with attractive stimuli during the intraspecific calling interactions, the stronger overlap avoidance, combined with the longer post-stimulus suppression and modal call delays will result in longer time periods between consecutive calls, which is exactly what we found in our data set on plasticity in call features. This shows that there is a trade-off between plasticity in call feature and call timing in *H. cinerea,* and that precise temporal presentation of the call (and subsequent avoidance of signal interference) may be more important for a male's attractiveness.

Reduced call period during call timing interaction may have another unexpected consequence – by decreasing a male's call duty cycle (the time period during which calls are produced) it should lower his energy expenditure during any given night, thus increasing the absolute number of nights he can participate in the chorus. This chorus attendance, more so than advertisement call features or body size/condition, has been revealed as the best predictor of male mating success in most anuran species tested so far (Holliday & Tejedo 1995, Martins 1993; Höbel 2000)

Not only was plasticity in signal timing important for maintaining attractiveness vis-à-vis simulated rivals, males also responded differently to signals that differed in relevance: they exhibited selective attention. During the intraspecific experiment, males avoided call interference more with the attractive than the unattractive stimulus in all treatments, and when confronted with attractive and unattractive calls in the same playback, they attended more to the attractive one. These observations are consistent with prior studies that examined the trade-off between call feature and call timing preferences in female *H. cinerea* (Höbel 2010). In terms of attractiveness, it is advantageous for a male to present the

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leading call at all times relative to another male. However if presenting the lagging call, doing so behind an unattractive high-frequency male does not reduce a male's success nearly as much as lagging behind an attractive low-frequency one (Höbel 2010). Thus in the conspecific context, selection should be strongest for avoiding being the lagger after an attractive call. During the interspecific experiments, males also exhibited selective attention during the mixed treatment. A study looking at the importance of cross-species call overlap on female *H. cinerea* preferences for the conspecific call revealed that females generally prefer the conspecific call, even if it is presented in lagging position relative to a heterospecific one (Höbel 2015). Selection on males to avoid call overlap should thus be stronger when males are confronted with conspecific than heterospecific call interference. Our data on male call timing behavior indeed mirrors these predictions across contexts.

The observation of signal timing in both experiments may also be evidence of a proposed trade off between signal timing and chorus attendance that has been documented in other acoustically advertising species. Specifically, as the number of stimuli that a focal male attempts to avoid interference with increases, his overall duty cycle decreases. If other males also exhibit indiscriminate attention to nearby neighbors in a chorus environment, a feed back loop may develop resulting in decreasing duty cycle and lower chorus attendance (see Marin-Cudraz and Greenfield 2016). In *H. cinerea*, chorus attendance is the strongest determinant of male mating success (Gerhardt et al 1987). Thus, selection favors the evolution of selective attention behavior by which a male can maximize both his duty cycle and the attractive presentation of calls (ie. avoiding discrimination from females for production of lagging calls via signal timing). While others have observed this as selective attention to the the loudest of an individual's neighbors

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(Marin-Cudraz and Greenfield 2016), it stands to reason that given a small local chorus group in which males (both conspecific and heterospecific) are presenting in near equal amplitude, (pers observ.) selection would favor attending to the more relevant or more competitive of these stimuli (attractive/conspecific).

Ultimately our observations demonstrate that there is indeed plasticity in signal timing behaviors- mediated by selective attention and plasticity in temporal call features- that serves as a mechanism by which males may stay competitive in dynamic chorus environments. These local interactions may help explain previously documented variation in signal timing behaviors of this species (Höbel 2011), and may contribute to the overall development and structure of the larger chorus. It would be of interest to examine how changes in local signal timing may affect large-scale chorus patterns over the duration of a single night, specifically as amplexed males remove themselves from the chorus. Here, the relative attractiveness and spatial location of particular male's nearest neighbors may fluctuate, which would alter his signal timing behavior. Similarly, it would be worth examining how variation in signal timing shifts throughout the breeding season, particularly as other anuran species reach the peaks or ends of their respective seasons. Further, comparison of the trade offs between plasticity in signal timing behaviors and plasticity in temporal call features in relation to chorus density and/or the presence or absence of heterospecifics may provide insight to changes in the strength of selection for plasticity in each throughout a breeding season. For example there may be particular times– for instance in mid to late summer when chorus density may be lowest– in which the reduction of intrasexual interactions results in much weaker selection on signal timing abilities, while maximizing the temporal attractiveness of the call itself is more

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advantageous. This scenario may allow for variation in signal timing ability to persist, while

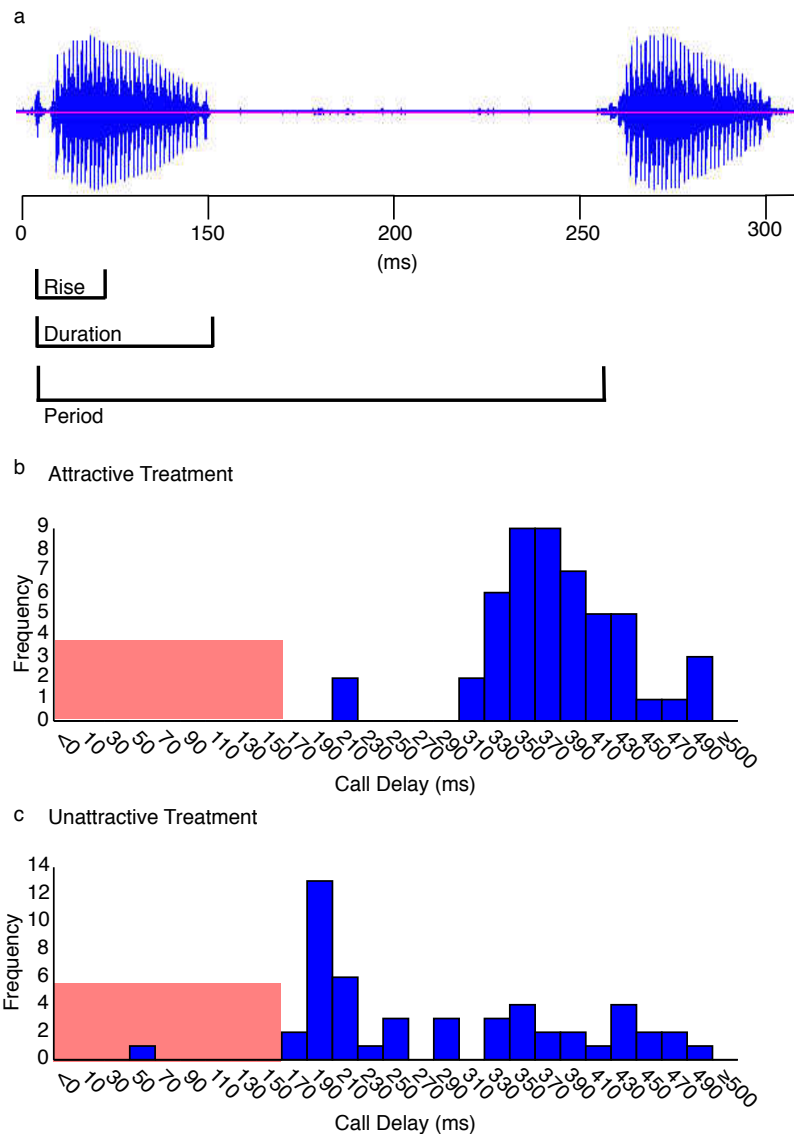simultaneously driving further evolution of plasticity in call features.

Figures and Tables

Figure 3.1 (a) Representative data from Male AA1 from the Intraspecific Experiment representing his temporal signal features, including call duration (total length of the call), call rise (time from the beginning of the call to the maximum amplitude of the call) and call period (the time from the beginning of one call to the beginning of the next call (measured in milliseconds). (b) A histogram of call delays during the attractive treatment and (c) unattractive treatment. Signal timing traits are obtained by first measuring call delay (time from stimulus onset to call onset), then using knowledge of the length of the stimuli to transform this data to call latency (time from stimulus offset to call onset). The shaded area represents the length of the stimulus, calling during which would produce an overlapped call. This data was used to determine the percentage of call overlap, modal delay, and post-stimulus suppression of a male's calls during each treatment.
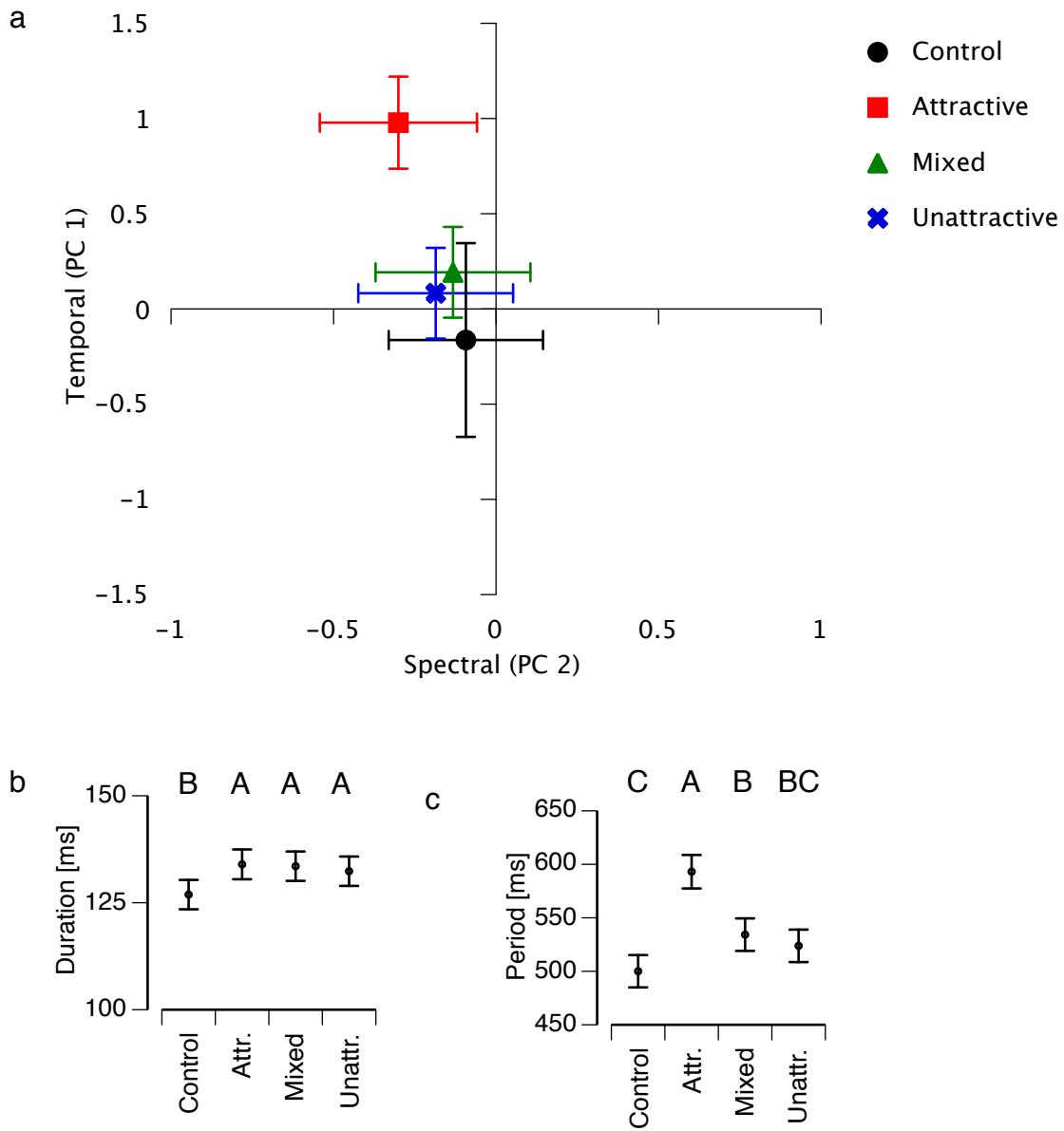
Figure 3.2 Effects of intraspecific experience on the signal features of *H. cinerea.*

(a) Shown are the principle components of male signal features for each treatment. PC1 loaded with the spectral components of the advertisement call (first and second frequency peak, while PC 2 loaded with the temporal parameters (call duration call period, and call rise time). A post-hoc student's *t* test revealed significant differences between treatments in the temporal call features of (b) call duration and (c) call period. Treatments not connected by the same letter are significantly different.

Figure 3.3 Effects of intraspecific experience stimuli on signal timing traits of (a) call overlap percentage (b) modal delay and (c) post-stimulus suppression of male calls. Treatments/Stimuli not connected by the same letter are significantly different.
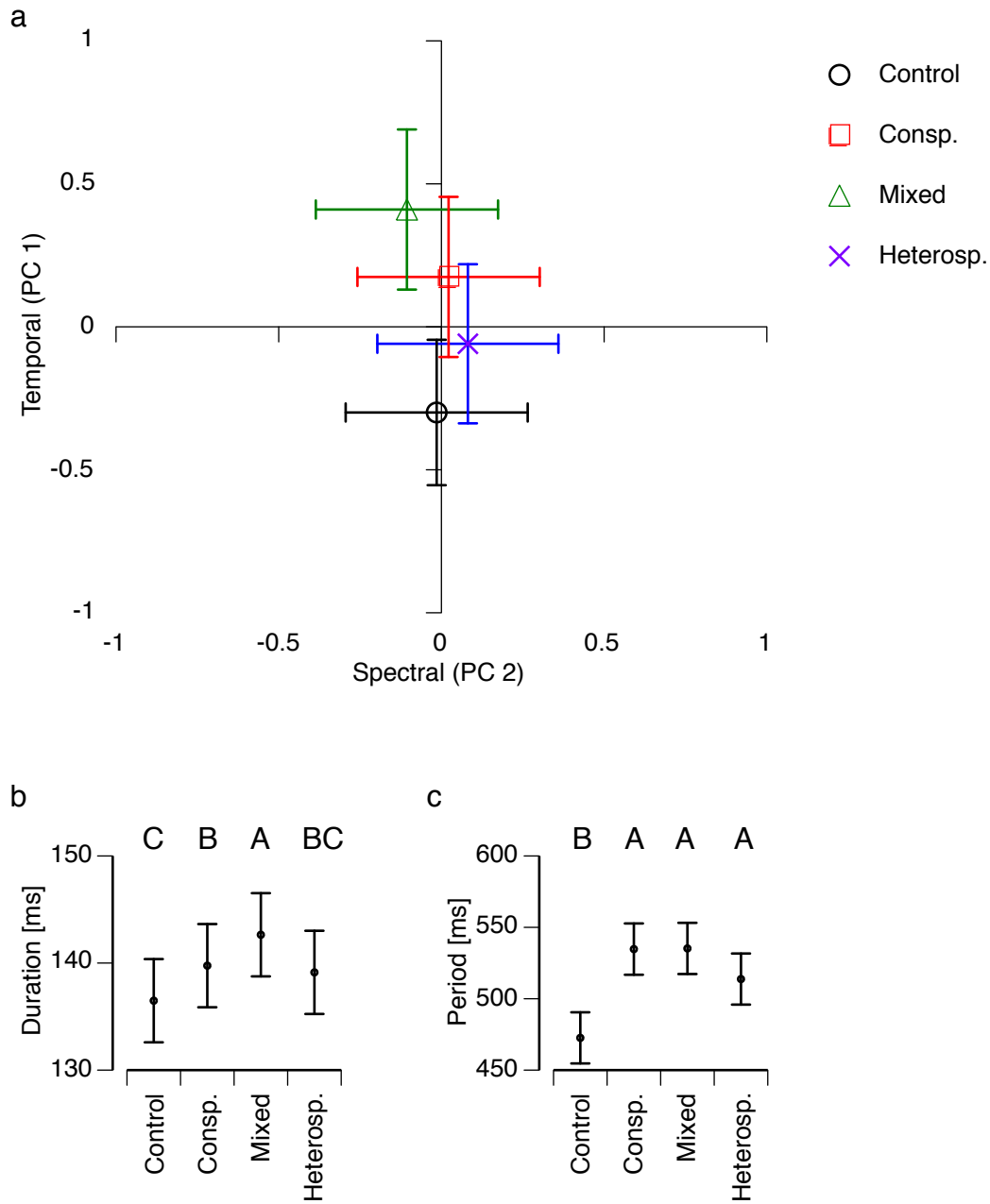
Figure 3.4 Effects of interspecific experience on the signal features of *H. cinerea*.

(a) Shown are the principle components of male signal features for each treatment. PC1 loaded with the temporal parameters (call duration call period, and call rise time,) while PC 2 loaded with spectral components of the advertisement call (first and second frequency peak.) A post-hoc student's *t* test revealed significant differences between treatments in the temporal call features of (b) call duration and (c) call period. Treatments not connected by the same letter are significantly different.
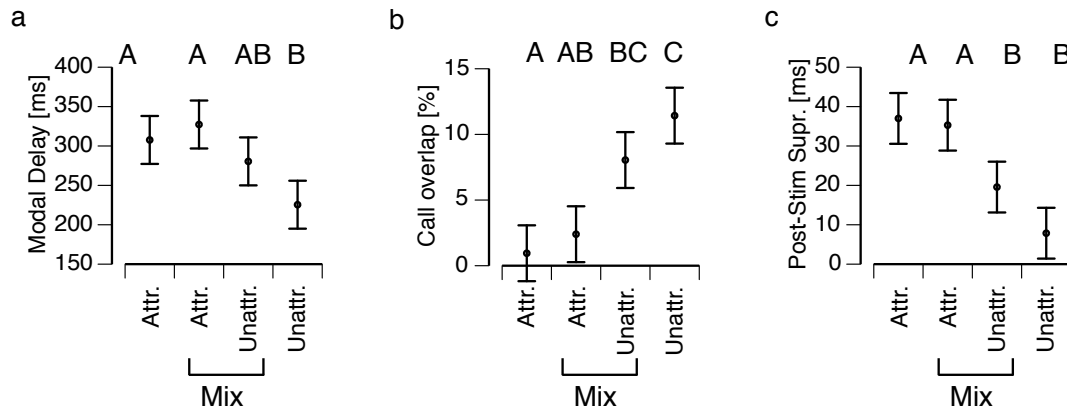
Figure 3.5 Effects of interspecific experience stimuli on signal timing traits of (a) call overlap percentage (b) modal delay and (c) post-stimulus suppression of male calls. Treatments/Stimuli not connected by the same letter are significantly different.
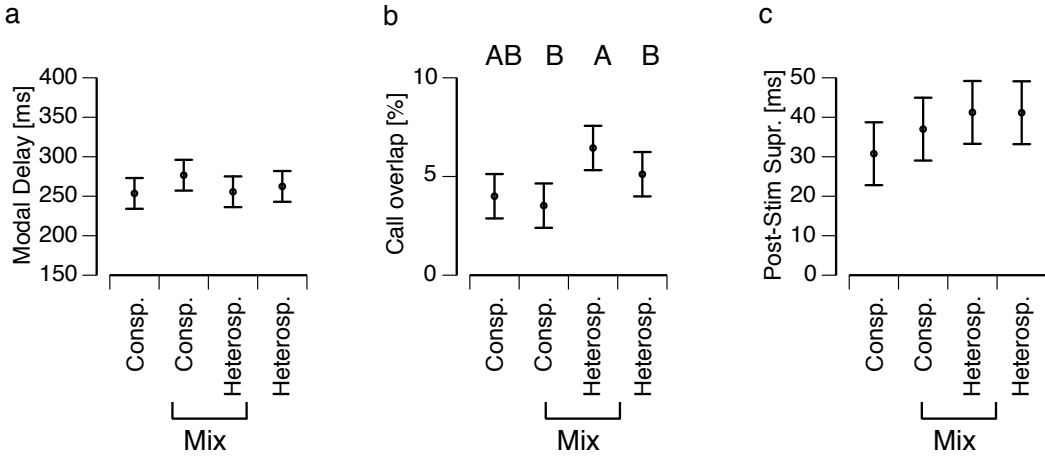
Table 3.1
Results of a mixed model (REML) examining the effects of intraspecific playback treatment, and temperature on variation in spectral (PC1) and temporal (PC2) characteristics of the advertisement call. Significant terms (P<0.05) are highlighted in bold.

	Spectral Parameters (PC1)			Temporal parameters (PC2)		
	df	F	P	df	F	P
Treatment	3,3	1.54	0.21	3,3	15.11	**<0.0001**
Temp	1,1	3.48	0.11	1,1	6.55	**0.0416**

Table 3.2
Results of a mixed model (REML) examining the effects of intraspecific playback treatment, and temperature on variation in call duration and call period. Significant terms (P<0.05) are highlighted in bold

	Duration			Period		
	df	*F*	*P*	*df*	*F*	*P*
Treatment	3,3	6.74	0.0002	3,3	9.36	**<0.0001**
Temp	1,1	2.20	0.15	1,1	1.99	0.18

Table 3.3
Results of a mixed model (REML) examining the effects of intraspecific playback treatment, stimuli within treatment, and temperature on variation in signal timing measures of modal delay, percentage overlap, and post stimulus suppression. Significant terms (P<0.05) are highlighted in bold.

Trait	Factor	df	F	P
Modal Delay	Treatment	2,2	3.71	**0.0448**
	Stimulus [Treatment]	1,1	1.71	0.21
	Temp	1,1	0.28	0.62
Percentage Overlap	Treatment	2,2	7.80	**0.0036**
	Stimulus [Treatment]	1,1	2.04	0.17
	Temp	1,1	9.06	**0.30**
Post Stimulus Suppression	Treatment	2,2	11.32	**0.0007**
	Stimulus [Treatment]	1,1	6.22	**0.0226**
	Temp	1,1	0.71	0.43

Table 3.4
Results of a mixed model (REML) examining the effects of interspecific playback treatment and temperature on variation in temporal (PC1) and spectral (PC2) characteristics of the advertisement call. Significant terms (P<0.05) are highlighted in bold

	Temporal parameters (PC1)			Spectral parameters (PC2)		
	df	*F*	*P*	*df*	*F*	*P*
Treatment	3,3	13.20	**<0.0001**	3,3	0.70	0.55
Temperature	1,1	2.54	0.1234	1,1	1.46	0.24

Table 3.5 Results of a mixed model (REML) examining the effects of interspecific playback treatment, and temperature on variation in call duration and call period. Significant terms (P<0.05) are highlighted in bold

	Duration			Period		
	df	F	P	df	F	P
Treatment	3,3	5.26	**0.0018**	3,3	13.98	**<0.0001**
Temperature	1,1	17.49	**0.0056**	1,1	6.43	**0.0427**

Table 3.6
Results of a mixed model (REML) examining the effects of interspecific playback treatment, stimuli within treatment, and temperature on variation in signal timing measures of modal delay, percentage overlap, and post stimulus suppression. Significant terms (P<0.05) are highlighted in bold.

Trait	Factor	df	F	P
Modal Delay	Treatment	2,2	0.32	0.73
	Stimulus [Treatment]	1,1	1.35	0.25
	Temp	1,1	5.67	**0.0365**
Percentage Overlap	Treatment	2,2	0.18	0.84
	Stimulus [Treatment]	1,1	4.58	**0.0391**
	Temp	1,1	0.03	0.88
Post Stimulus Suppression	Treatment	2,2	0.61	0.55
	Stimulus [Treatment]	1,1	0.17	0.68
	Temp	1,1	5.09	**0.0455**

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<div align="center">**Curriculum Vitae**</div>

Education:
University of Wisconsin-Milwaukee, Milwaukee, WI (May 2018)
- Ph.D. Biological Sciences: Behavioral Ecology

Vanderbilt University College of Arts and Science, Nashville, TN 2009
- B.A. Biological Sciences: Evolution, Ecology, and Organismal Biology
- Minor: Italian Language

Research Experience:
Doctoral Research: University of Wisconsin-Milwaukee. Advisor: Dr. Gerlinde Hoebel
- Laboratory study of foraging techniques of Green Tree Frogs, *Hyla cineria.*
- Field study of the role of behavioral plasticity in the mating behavior of Green Tree Frogs.

Assisted Research Projects:
- Laboratory study of behavioral plasticity in response to light pollution in the gray tree frog, *Hyla versicolor*
- Laboratory study of handedness in Oriental Fire-bellied Toad, *Bombina Orientalis*
- Field study of Intrasexual interactions in the Grey Tree Frog, *Hyla versicolor*.
- Field study of the visitation behavior of bumble bees (*Bombus* species)

Teaching Experience:
Lead Instructor: Introduction to Zoology Laboratory
      University of Wisconsin-Milwaukee 2010-2017
- Revised and published a new student lab manual (2012, 2014)
- Instruct weekly lectures and dissections
- Provide regular student assessment via weekly quizzes and homework assignments
- Create and hold midterm and final laboratory practical exams
- Proctor lecture exams

Adjunct Instructor
    Milwaukee Institute of Art and Design 2016
- Animal Communication (1 semester)
- Introductory Biology (2 semesters)

Work Experience
- Laboratory Coordinator 2016-2017
  - Tested and repaired recording and playback equipment
  - Coordinated schedules of undergraduate research assistants
  - Advised on experimental design and implementation

Research Interests:

- Theoretical and field study of the dynamic role of sexual selection in changing communities
- Exploration of the behavioral changes of captive animal populations, specifically those part of reintroduction programs.
- Plasticity in animal communication in response to the social environment

Publications
- G., Kim, Diana., Neelon, Daniel. 2015. Do green treefrogs eavesdrop on prey calls? Journal of Herpetology 48:389-393.

- Neelon, D.P. and Höbel, G., 2017. Social plasticity in choosiness in green tree frogs, *Hyla cinerea*. Behavioral Ecology, 28:1540-1546.

Presentations
- Hoebel, G., Kim, D., Neelon, D. 2011. *Green Tree Frogs do not eavesdrop on prey signals*. National Meeting of the Animal Behavior Society Poster Presentations

- Neelon, D. Höbel G. 2013. *Plasticity in female mate choice preferences in response to novel heterospecific male signals*. Riveredge Nature Center Student Symposium. November 9th, 2013

- Neelon D. Höbel G. 2014. *Dynamic signaling environments reveal plasticity in female mate choice.* University of Wisconsin-Milwaukee Biological Sciences Research Symposium April 25, 2014

Community Outreach
- Boy Scouts of America – Night Nature Hike
     May 18th 2012 San Marcos, TX
- Milwaukee River Advocates: Creatures of the Night Nature Walk
     September 22nd 2012 Milwaukee, WI