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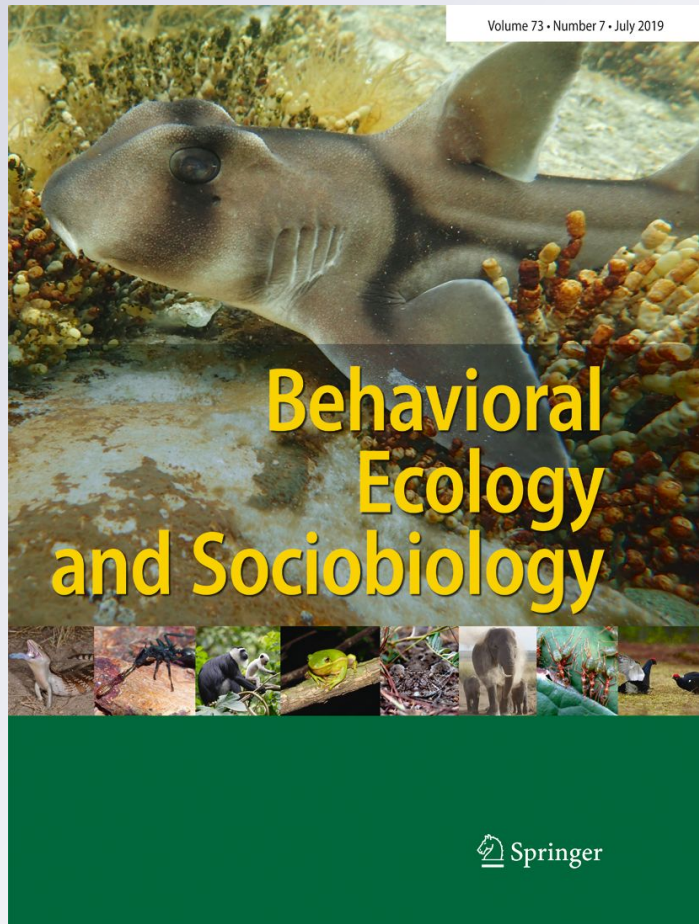
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Staying ahead of the game—plasticity in chorusing behavior allows males to remain attractive in different social environments

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Abstract

The dynamic nature of many breeding aggregations, where the composition and attractiveness of a male's competitors are ever changing, places extreme pressure on advertising males to remain competitive. In response to this challenge, males may adjust the properties of their calls or change when they signal relative to their nearest neighbors, which are likely their strongest competitors. We used two playback experiments—one simulating a conspecific environment and the other simulating a mixed-species environment—to test the hypothesis that males use social plasticity in signal features, signal timing, or both, to remain attractive. Further, we examined whether this plasticity is mediated by selective attention, through which males change calling behavior in response to the most relevant competitors, while disregarding less relevant rivals. We find that males change some temporal call features, but rely strongly on signal timing to remain attractive relative to rivals. Simultaneous assessment of both types of calling plasticity allowed us to make sense of counterintuitive responses of male calling behavior that would otherwise appear non-adaptive. We further show that this plasticity is most pronounced in response to attractive/conspecific males. We discuss how sexual selection by female choice may influence the trade-off between call feature and call timing plasticity, as well as how competitive interactions on a local scale may affect the overall acoustic environment in the chorus.

Significance statement

Males of group-signaling species face intense pressure to stand out from the crowd to attract mates—not only must they produce attractive signals, those signals must also be perceived clearly above the din. As signaling is a costly endeavor, it would be adaptive for males to recognize the relative attractiveness of their competitors and to adjust signaling behavior as the social environment changes. Using playback experiments, we show that male treefrogs modify call features and call timing in response to more attractive rivals. Our study also highlights that when studying chorusing species, the dual demands of producing attractive calls and placing them in attractive positions require simultaneous attention to both aspects of calling behavior. Only then is it possible to appreciate the potential trade-offs involved: males lengthening their call period when interacting with attractive rivals would appear maladaptive without the knowledge that this behavior results in reduced call overlap.

Keywords Social plasticity · Sexual selection · Call timing

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Introduction

In the cacophony 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 advertisement signals, 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). Given the high cost of signaling, both in terms of energy expenditure and exposure to predators and parasites (Ryan et al. 1982; Prestwich et al. 1989), selection should favor males with the ability to adjust their signaling behavior in response to variation in the social environment.

To remain competitive, males may respond to the variability of the social environment broadly in two ways. First, a male may exhibit plasticity in his *signal 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 advertisement 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, to become more attractive than a nearby rival (Lopez et al. 1988; Wagner 1989; Bee et al. 2000). Second, a male may show plasticity in *signal timing* (Buck 1938; Grafe 1999; Greenfield 2015), often specifically in a way that reduces signal interference (Klump and Gerhardt 1992; Schwartz 1993; Greenfield 2015). Such changes in signal timing can involve very different time frames, ranging from chorus initiation at different hours of the night to avoid interference from heterospecifics (Greenfield 1993) to alternation of individual signals or even individual signal elements (Grafe 2003; Martinez-Rivera and Gerhardt 2008). One benefit of precisely timed signals is to ensure that they 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 et al. 1997; Snedden 1998; Greenfield and Snedden 2003; but see Grafe 1999). In these cases, females tend to discriminate against signals that are perceived to follow a leading signal. This may be a signal that (1) overlaps with the end of the leading signal or (2) follows the leading signal too closely (within the “forbidden interval”, Greenfield et al. 1997).

Not surprisingly, males of these species generally do not place their signals in such unattractive positions (Greenfield et al. 1997; Snedden and Greenfield 1998; Greenfield and Rand 2001; Greenfield and Snedden 2003; Höbel 2011).

Although altering one's signaling behavior in response to changes in social competition seems beneficial, it may also carry costs (Wells and Taigen 1986; Marin-Cudraz and Greenfield 2016). For example, trying to avoid signal overlap with every single rival within the breeding aggregation simultaneously would greatly reduce a male's amount of signaling (Greenfield and Rand 2001). Rather, males may maximize the efficiency of signaling behavior by attending only to their closest rivals, to which a mate-searching female will most

likely compare them (Grafe 1996; Greenfield and Snedden 2003; Reichert and Gerhardt 2013). There is evidence that males do exhibit such *selective attention* in signal timing behavior, by adjusting to up to three of their loudest (i.e., nearest) neighbors (Narins 1992; Greenfield 1994a, b; Greenfield and Rand 2001). Whether males are also able to distinguish the relative attractiveness of their nearest neighbors, and then preferentially attend to the most competitive rival while disregarding others, is currently unknown.

We examined social plasticity in calling behavior in a treefrog, testing whether males show plasticity in call features, call timing, or both. We additionally examined whether social plasticity was modulated by selective attention, and whether changes in calling behavior were more pronounced when males were confronted with more attractive rivals. To our knowledge, this is the first study to assess both types of signaling plasticity simultaneously, allowing for a direct comparison of the magnitude at which males modify their call features and call timing, as well as revealing whether selective attention is an important component of both behaviors. The hypothesis that males use plasticity in *call features* to remain competitive makes the following predictions: (i) calls should be different when males call alone compared to when they interact with rivals; (ii) males should change their call features in ways that increase their attractiveness to females; and (iii) if selective attention is an important component of this plasticity, then the greatest increase in call attractiveness should be elicited by signals that represent stronger competition (attractive or conspecific stimuli, respectively). The hypothesis that males use plasticity in call *timing* to remain competitive makes the predictions that (i) males should overlap fewer calls than expected by chance and (ii) if mediated by selective attention, then males should time their own calls more precisely when confronted with more competitive signals.

Methods and materials

Study species and study site

To test these hypotheses, we focused on the calling behavior of the Green Treefrogs, *Hyla cinerea* (Anura: Hylidae), a common anuran species found throughout the southeastern USA (Conant and Collins 1998). Each night from April through July, males congregate in choruses at ponds and swampy areas, where they produce advertisement calls which attract females. The calls are short, ranging from 100 to 200 ms in duration, with a repetition rate of 50–80 times per minute. Calls contain two spectral peaks, one in the low-frequency range (0.68–1.2 kHz) and one in the high-frequency range (2.3–3.7 kHz); the peak in the low-frequency range is often, but not always, more intense (i.e., dominant frequency) (GH unpubl. data from recordings of $n = 548$ males across the

species range). Females show preferences for a number of male call features, including call frequency, call duration, call amplitude, and call repetition rate (Gerhardt 1974, 1981, 1987; Höbel 2010). Females are also very attentive to call timing, preferring the leading of two calls that are overlapping or perceived in close succession (Höbel and Gerhardt 2007; Höbel 2010).

The range of *H. cinerea* overlaps with, and populations may share breeding ponds with, several other hylid species of the same genus (*H. versicolor*, *H. chrysoscelis*, *H. gratioiosa*, *H. squirella*; DN, GH pers. obs; Gerhardt 1974; Gordon et al. 2017). *H. cinerea* may call in single- or multi-species aggregations, and due to between-species differences in habitat preferences and timing of breeding season, chorus composition can vary substantially between nights (DN and GH pers. obs.).

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. It was not possible to record data blind because our study involved focal animals in the field.

Testing male signaling plasticity

To test for plasticity in call feature and call timing behavior, we confronted calling male frogs with playbacks that simulated social environments that varied in the composition and attractiveness of potential rivals. We conducted two experiments: (i) *intraspecific*—featuring only conspecific playbacks with simulated rivals that differed in attractiveness—and (ii) *interspecific*—featuring conspecific as well as heterospecific playbacks (i.e., mixed-species chorus), where we consider the conspecific to be the more attractive stimulus.

Stimulus generation

Synthetic calls of *H. cinerea* consist of one frequency component in the low-frequency range and two frequency components in the high-frequency range (Gerhardt 1987; Höbel 2010). We generated a number of synthetic advertisement calls using a custom-written DOS program courtesy of J.J. Schwartz. We then used Audacity® software (Audacity Team 2013) to create treatment playbacks that we presented to males at the pond. For the conspecific playback treatment, we copied a number of synthetic *H. cinerea* calls into a longer file, and for the interspecific mixed treatment, we copied *H. cinerea* and *H. gratioiosa* calls together. 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 plasticity in calling behavior in the face of intraspecific social competition, we generated stimuli representing variation in relative attractiveness of conspecific competitors. We simulated this variation using playbacks varying in call frequency (pitch), the call trait for which females have the strongest preference (Gerhardt 1987; Höbel 2010). 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). All call stimuli had a duration of 160 ms, a call rise time of 25 ms, and a fall time of 50 ms. These specific synthetic calls have previously been shown to be attractive and unattractive to females of this population (Neelon and Höbel 2017). Incorporating knowledge about variation in chorus density, call period, and call timing behavior (Höbel and Gerhardt 2007; GH pers. obs.), we created playbacks that simulated the presence of several interacting males. To do this, we copied the synthetic call stimuli together and varied the duration of the silent intervals between them. Silent intervals were 300, 400, or 500 ms long, and haphazardly interspersed between call stimuli following the rule that a given interval duration could not follow itself more than twice in a row. The two extremes of the interval range were chosen to mimic a chorus environment in which some males called singly (represented by longer intervals) and others antiphonally with other males (represented by shorter intervals). Since we could not simulate the presence of several males by varying call features (as this would have changed our treatments, that were based on denoting male attractiveness through differences in call frequency), our only option was to vary inter-call intervals in ways that would be too short or too long to be produced by one or two males.

To generate the treatment simulating an attractive social environment, the stimulus playback only contained the attractive 800 Hz call; the unattractive treatment only contained the unattractive 1100 Hz 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). We achieved identical temporal stimulus presentation by generating a sequence of inter-call intervals for one of the treatments, and then copying this sequence when generating the other treatments.

Interspecific experiment

To test the hypothesis that plasticity in calling behavior results in the maintenance of attractiveness to females in a social environment that also contains heterospecifics, we confronted calling males with calls of conspecifics as well as those of Barking Treefrogs (*H. gratioiosa*). We chose this species as the heterospecific stimulus because their calls have similar

acoustic structure; call duration is very similar, and although Barking Treefrogs calls are lower in frequency, there is substantial overlap in the frequency range between the calls of both species (Oldham and Gerhardt 1975).

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. gratiiosa* call (0.5 + 1.5 + 2.0 kHz) (Oldham and Gerhardt 1975; Gerhardt 1981; Höbel and Gerhardt 2003). Temporal parameters were identical between the two stimuli: call duration was 150 ms and call rise and fall times were set to 25 ms and 50 ms, respectively.

To generate the treatment simulating a conspecific social environment, the stimulus playback only contained *H. cinerea* calls; the heterospecific treatment only contained *H. gratiiosa* calls (with identical temporal presentation of calls). To generate the mixed treatment, we alternated the *H. cinerea* stimulus with the *H. gratiiosa* stimulus, resulting in a 1:1 ratio of call types (again, identical temporal presentation of calls). During these playbacks, the silent intervals between consecutive call stimuli varied between 180 and 1800 ms (average 900 ms). We used ten different interval durations (increasing in steps of 180 ms) and haphazardly interspersed them between call stimuli following the rule that a given interval duration could not follow itself more than twice in a row. The lower average stimulus presentation rate (compared to the intraspecific experiment, see above) reflects the lower call rate typical for *H. gratiiosa*.

Experimental procedure We conducted the conspecific experiment in 2012 and the heterospecific experiment in 2013. Male playback trials were conducted during times when the chorus was most active (2100–2400 h). We chose focal males that 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 positioned 1 m away from the calling male. We used a Marantz PMD670 digital recorder and a Sennheiser K3-ME88 microphone to record the focal males.

We tested plasticity in chorusing behavior in a repeated measures design. At the beginning of each trial, the spontaneous calling of the focal male was recorded for 2 min. Then, we started to broadcast the playback treatments. Playback treatments lasted 5 min each, followed by 2 min 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/heterospecific/mixed, respectively). At the end of each playback trial,

we measured ambient air temperature at the male's calling location (all males called from land.)

Call analysis

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 call: (i) low-frequency peak (usually the third harmonic) and (ii) high-frequency peak (usually the ninth or tenth harmonic), either of which may be the dominant frequency, (iii) call duration, (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). We measured five calls (selected for optimal signal-to-noise ratio) from the middle portions of 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 call feature plasticity maintains attractiveness to females across 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 call timing behavior (Fig. 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 sequential 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 sequential 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). First, 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 shorter than this value corresponds to a call given in an overlapped, lagging position. We focused on overlapped lagging calls because these are strongly discriminated against by females (Höbel and Gerhardt 2007; Höbel 2010) (Fig. 1c).

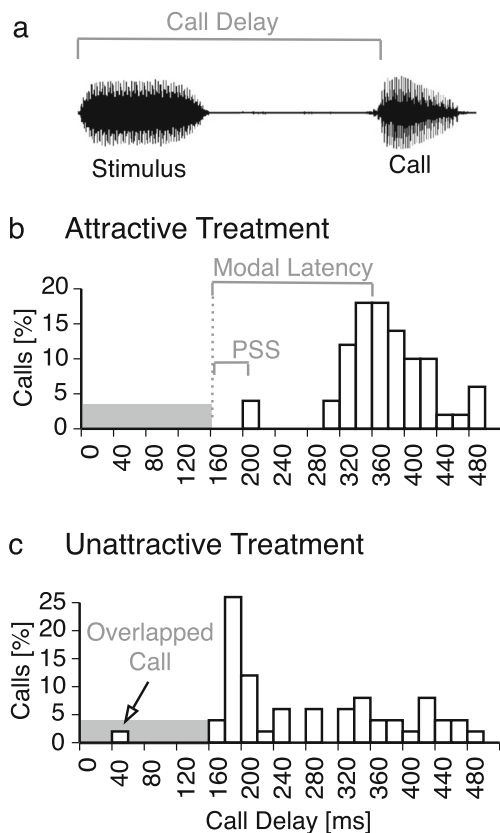


Fig. 1 **a** Representative waveform recording from the intraspecific experiment showing a stimulus followed by an advertisement call (male AA1). A histogram of call delays during the **b** attractive treatment and **c** unattractive treatment. Signal timing traits are obtained by first measuring call 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 gray 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 latency, and post-stimulus suppression (PSS) of a male's calls during each treatment

Second, 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 initiate their own calls (Höbel and Gerhardt 2007). We did not measure call latency directly because the amplitude envelope of the *H. cinerea*/*H. gratiosa* call (and their synthetic equivalent), with its steep onset but relatively shallow offset, makes it 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 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 *the modal call latency* (i.e., the timing at which the male placed most of its calls) (Fig. 1b, c). We chose the mode rather than the mean, 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 neurophysiological, 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.

Statistical analysis

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

Analysis of call feature plasticity Since calls are multivariate traits, and the constituent call properties may be correlated with each other, we first ran a principal component analysis and used this information to guide our subsequent statistical analysis of call features.

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 may affect call properties in frogs (Gerhardt and Huber 2002), and we entered male ID to account for each male contributing five call measurements per treatment. For testing our hypothesis, we are only interested in the effect of treatment, and for simplicity, we only report the statistical results for this term.

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 rise time, call duration, and call period, respectively, as response variables, treatment and temperature as test variables, and male ID as a random term. Again, since for testing our hypothesis, only the treatment term is of interest, we only report the statistical results for treatment.

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 behaviors in response to different social environments. We entered

percentage call overlap (arcsine square root transformed before analysis), post-stimulus suppression and modal call latency, respectively, as the response variables, and treatment (i.e., mixed) and stimulus type (i.e., attractive/unattractive; nested within treatment) test variables. In addition, we entered temperature as another test variable to account for potential temperature effects on call timing behavior and male ID as a random term to account for the same male contributing two data points in the mixed treatment. Note that because frogs time their calls in response to immediate signal interference, the important comparison for testing our hypothesis is not always the treatment term. Particularly in the mixed treatment, and to evaluate selective attention, the stimulus type term is more relevant.

Effect sizes

We calculated effect sizes to compare the magnitude of calling plasticity between experiments, treatments, and behavior types (call features vs call timing). We first calculated Cohen's *d* by using mean values and a pooled standard deviation between treatments, and then calculated the correlation coefficient *r* from Cohen's *d* (Cohen 1988). Values of *r* range from 0 to 1 and have similar interpretations as *r*² in a simple linear regression. Correlation values that range from 0 to 0.3 indicate small effect sizes, 0.3–0.5 indicate intermediate effect sizes, and values greater than 0.5 indicate large effect sizes.

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 occasionally ceased calling during some treatments.

Intraspecific experiment

Call plasticity

The principal 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. PC2 had an eigenvalue of 1.31 and loaded mainly with the three temporal call parameters. Together, the two first PCs accounted for 69.9% of the variation.

The mixed model identified treatment as a significant determinant of variation in PC2, the principal component that correlated with temporal call parameters (PC1: $F_{3,139} = 1.54$, $p = 0.21$; PC2: $F_{3,139} = 15.11$, $p < 0.0001$; Fig. 2). Further analysis focusing on individual temporal traits revealed significant effects of treatment on call duration ($F_{3,144} = 5.26$, $p = 0.002$) and call period ($F_{3,142} = 13.98$, $p < 0.0001$);

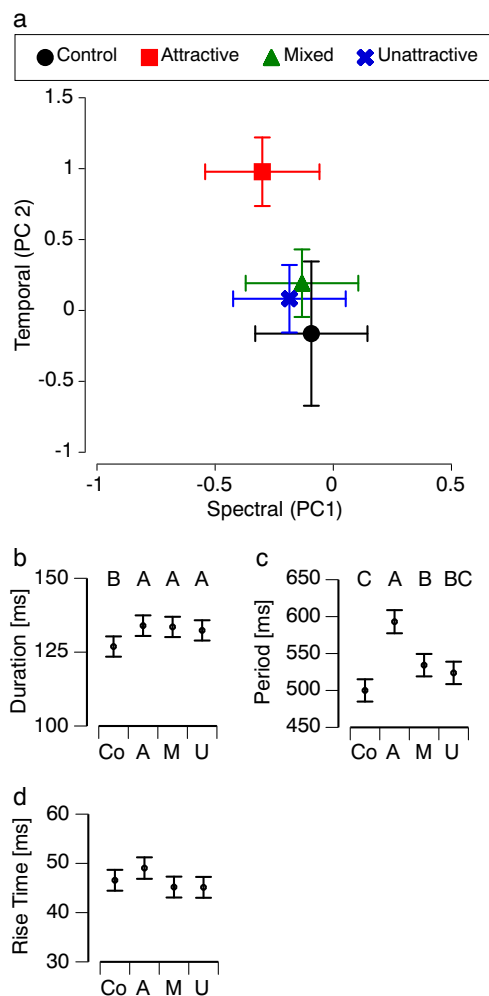


Fig. 2 Effects of intraspecific experience on the signal features of *H. cinerea*. **a** Shown are the principal components of male signal features for each treatment. A *post hoc* Student's *t* test revealed significant differences between treatments in the temporal call features of **b** call duration, **c** call period, and **d** call rise time. Shown are least square means and standard error. Treatments not connected by the same letter are significantly different. "Co" indicates the control; "A" indicates the attractive treatment; "U" indicates the unattractive treatment; "M" indicates the mixed treatment

compared to the control, during which males called alone, call duration increased across all playback treatments (Fig. 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 (Fig. 2c). Call rise time was unaffected by the playback treatments ($F_{3,144} = 1.95$, $p = 0.12$, Fig. 2d).

Call timing

All three measures of call timing behavior were affected by the presented stimulus type (Table 1, Fig. 3). Males overlapped fewer calls with the attractive stimulus than the unattractive one (Fig. 3a), and both post-stimulus suppression

Table 1 Results of a mixed model (REML) examining the effects of intraspecific playback treatment, and stimulus type within treatment, on variation in signal timing measures of modal delay, percentage overlap, and post-stimulus suppression. Significant terms ($P < 0.05$) are highlighted in italics

Trait	Factor	df	F	P
Modal latency	Treatment	2,18	3.71	<i>0.0448</i>
	Stimulus [treatment]	1,18	1.71	0.21
Percentage overlap	Treatment	2,18	7.80	<i>0.0036</i>
	Stimulus [treatment]	1,18	2.04	0.17
Post-stimulus suppression	Treatment	2,18	11.32	<i>0.0007</i>
	stimulus [treatment]	1,18	6.22	<i>0.0226</i>

(Fig. 3b) as well as modal call latency (Fig. 3c) were longer after attractive compared to unattractive stimuli. Moreover, in the mixed treatment, males changed their call timing behavior more strongly in response to the attractive compared to the unattractive stimulus (Fig. 3a–c), although this only reached statistical significance for post-stimulus suppression.

Interspecific experiment

Call plasticity

The principal 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. PC2 had an eigenvalue of 1.22 and loaded mainly the spectral parameters. Together, the two PCs accounted for 56.4% of the variation.

The mixed model identified treatment as a significant determinant of variation in the principal component that correlated with temporal call parameters (PC1) (PC1: $F_{3,254.8} = 13.2$, $p < 0.0001$; PC2: $F_{3,255.4} = 0.7$, $p = 0.55$; Fig. 4). Further analysis focusing on individual temporal traits revealed significant effects of treatment on call duration ($F_{3,280.6} = 6.74$, $p = 0.0002$), call period ($F_{3,277.3} = 9.36$,

$p < 0.0001$), and call rise time ($F_{3,281.4} = 3.36$, $p = 0.02$): call duration and call rise time significantly increased during the conspecific and mixed treatments relative to the control, with call duration showing the greatest increase during the mixed treatment (Fig. 4b, d). Call period increased relative to the control across all treatments (Fig. 4c).

Call timing

Only call overlap was significantly affected by the presented stimulus type (Table 2, Fig. 5). Males overlapped fewer calls with the conspecific stimulus than with the heterospecific one (Fig. 5a) during the mixed treatment.

Effect sizes

Comparison of effect sizes of the changes in male calling behavior across experiments, treatments, and behavior types (call features vs call timing) shows three interesting patterns (Table 3). First, effect sizes were generally larger for calling behaviors associated with call timing rather than call features (particularly in the intraspecific experiment). Second, effect sizes were generally larger when males interacted with the attractive rather than the unattractive call, or the conspecific rather than the heterospecific one, respectively. And third, when looking at the same behavioral response (i.e., change in call period or difference in % call overlap), effect sizes are generally larger in the intraspecific compared to the interspecific experiment. The latter two observations suggest that males show selective attention and change calling behavior more when confronted with more relevant (attractive/conspicuous) rivals.

Discussion

Male *H. cinerea* showed plasticity in both call features and in call timing in response to variation in the social environment.

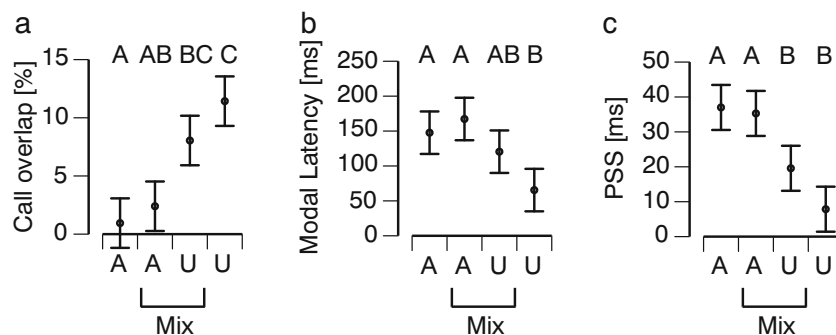


Fig. 3 Effects of intraspecific experience stimuli on signal timing traits of a call overlap percentage, b modal latency, and c post-stimulus suppression (PSS) of male calls. Shown are least square means and standard error. Treatments/stimuli not connected by the same letter are significantly

different. “A” indicates the attractive stimuli; “U” indicates the unattractive stimuli; “Mix” indicates data from the mixed treatment that presented conspecific and heterospecific stimuli together

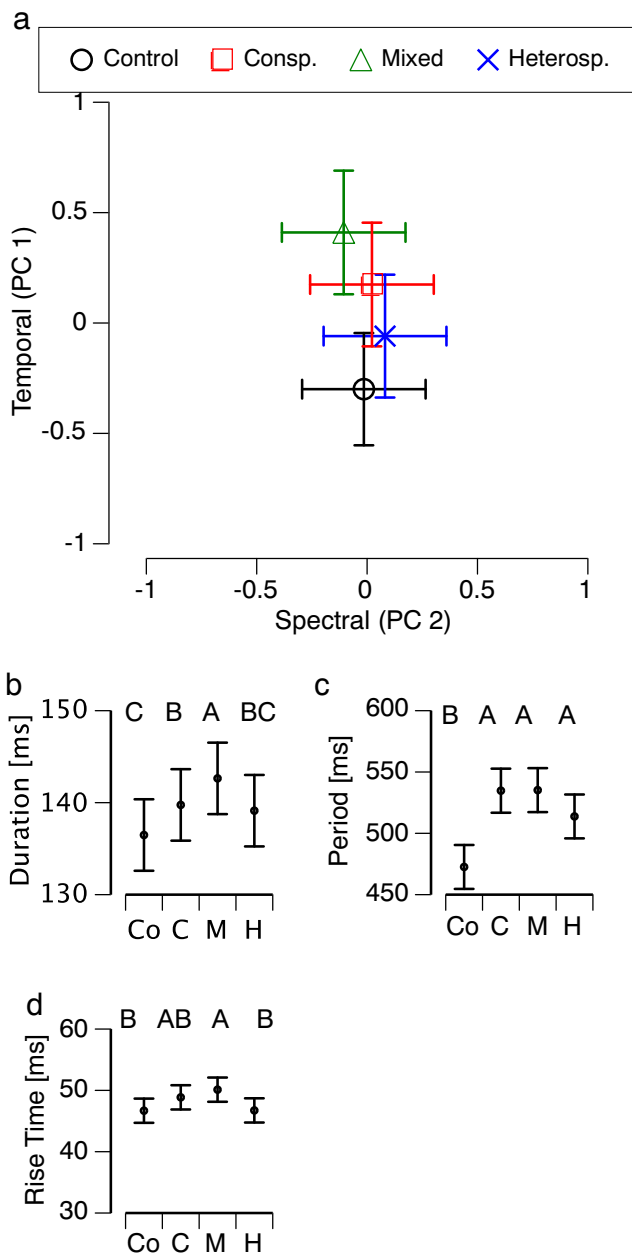


Fig. 4 Effects of interspecific experience on the signal features of *H. cinerea*. **a** Shown are the principal components of male signal features for each treatment. Consp. = conspecific treatment; Heterosp. = heterospecific treatment. A *post hoc* Student's *t* test revealed significant differences between treatments in the temporal call features of **b** call duration, **c** call period, and **d** call rise time. Shown are least square means and standard error. Treatments not connected by the same letter are significantly different. "Co" indicates the control; "C" indicates the conspecific treatment; "H" indicates the heterospecific treatment; "M" indicates the mixed treatment

Moreover, males were very attuned to the attractiveness of the simulated rivals: (i) calling plasticity was more evident during the intraspecific than during the interspecific experiment and (ii) when simultaneously confronted with rivals that differed in attractiveness (mixed treatments in each experiment), males

Table 2 Results of a mixed model (REML) examining the effects of interspecific playback treatment, and stimulus type within treatment, on variation in signal timing measures of modal delay, percentage overlap, and post-stimulus suppression. Significant terms ($P < 0.05$) are highlighted in italics

Trait	Factor	<i>df</i>	<i>F</i>	<i>P</i>
Modal latency	Treatment	2,36	0.32	0.73
	Stimulus [treatment]	1,36	1.35	0.25
Percentage overlap	Treatment	2,36	0.18	0.84
	Stimulus [treatment]	1,36	4.58	<i>0.0391</i>
Post-stimulus suppression	Treatment	2,36	0.61	0.55
	Stimulus [treatment]	1,36	0.17	0.68

showed selective attention by attending primarily to the more relevant (attractive/conspecific) stimulus.

Female *H. cinerea* show preferences for a number of call traits, including longer call duration and shorter call period. The strongest preference in this species, however, is for call frequency (Gerhardt 1987), and in our study population, males with lower-frequency calls are more attractive (Neelon and Höbel 2017). This begs the question of why we did not observe plasticity in spectral call properties. Some frog species are indeed capable of changing call frequency (Lopez et al. 1988; Wagner 1989; Bee et al. 2000), but in most species, spectral call properties seem to be largely determined by the morphology of the call production mechanism (McClelland et al. 1996). *Hyla cinerea* may belong to this latter group, and males may not be physiologically capable of modulating their spectral call traits. Temporal call properties tend to be more plastic in frogs (Gerhardt and Huber 2002), and males in our study did show more plasticity in these traits. Males did increase call duration during playback trials (compared to the controls), which should increase their attractiveness to females. However, they also increased call period, which should make them less attractive to females.

At first glance, increasing call period is a counterintuitive response to social competition. However, it is the logical outcome arising from the strong plasticity in call timing we observed during the playback trials. This is likely associated with the inhibitory-resetting mechanism, which reduces call overlap with other males. Here, the calling male's central call rhythm generator produces rhythmic calling by rising from basal to trigger level, upon which production of a call is initiated, after which the generator returns to basal level and the process repeats. Call-timing adjustments occur when the perception of a rival's call resets this generator and remains inhibited at basal level until the end of that call, upon which it rebounds and triggers the focal male's next call (Greenfield et al. 1997; Höbel and Gerhardt 2007). Thus, the affected call period is normally longer than during free-running calling without interruptions (Greenfield et al. 1997).

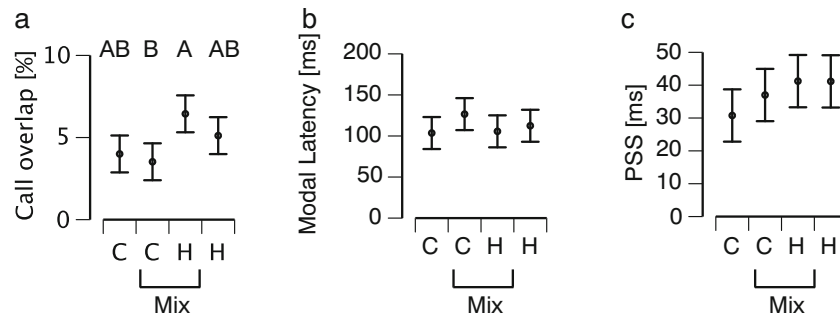


Fig. 5 Effects of interspecific experience stimuli on signal timing traits of male calls. Shown are least square means and standard error. Treatments/stimuli not connected by the same letter are significantly

different. “C” indicates conspecific stimuli; “H” indicates heterospecific stimuli; “Mix” indicates data from the mixed treatment that presented conspecific and heterospecific stimuli together

Faced with a trade-off between plasticity in call features and call timing, male *H. cinerea* seem to emphasize the precise timing of the call. This is an adaptive strategy, because in female *H. cinerea*, preferences for call period are relatively weak, while call timing preferences are quite strong and frequently override preferences for call features (Höbel 2010). Further, increasing call period in order to achieve more precise call timing may even have a positive outcome for the male: decreasing a male’s call duty cycle (the amount of call energy produced per unit time) 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 many frogs and toads, including *H. cinerea* (Gerhardt et al. 1987; Martins 1993; Halliday and Tejado 1995; Höbel 2000).

Selective attention for rival attractiveness

Not only was there plasticity in calling behavior vis-à-vis simulated rivals, males also responded differently to stimuli that differed in relevance: they exhibited selective attention.

Selective attention has been documented for a number of group-signaling taxa, but has previously been restricted to attention based on call amplitude (i.e., males attending to the loudest neighbors; Greenfield and Rand 2001; Greenfield and Snedden 2003; Marin-Cudraz and Greenfield 2016). Here, we show, for the first time, that selective attention can also be based on signal attractiveness via spectral call properties.

In addition, males not only selectively avoided call overlap with certain stimuli relative to others, but more fine-scale aspects of call timing, such as call suppression and call delays, sometimes changed as well. The signal integration times associated with the extremely short time intervals during call timing adjustments (i.e., post-stimulus suppression lifts after 20–40 milliseconds; Figs. 3 and 5; Höbel and Gerhardt 2007) suggest that male *H. cinerea* may have surprisingly sophisticated signal processing abilities that merit detailed neurophysiological investigation.

During the intraspecific experiment, males avoided call interference more with the attractive than the unattractive stimulus, including the mixed treatment that confronted them with attractive and unattractive calls in the same playback. This is consistent with a prior study that examined the trade-off between call feature and call timing preferences in female

Table 3 Effect sizes of changes in call features and call timing in response to different call stimuli. Values that range from 0 to 0.3 indicate small effect sizes, 0.3–0.5 indicate intermediate effect sizes, and values greater than 0.5 indicate large effect sizes

	Intraspecific experiment			Interspecific experiment		
Call features	Control/Attr	Control/Unattr	Attr/Unattr	Control/Consp	Control/Heterosp	Consp/Heterosp
Call duration	0.299	0.150	0.104	0.031	0.024	0.005
Call period	0.796	0.597	0.758	0.301	0.230	0.095
Call rise time	0.298	0.088	0.290	0.154	0.003	0.144
Call timing	Attr/Unattr.	Mix Attr/mix Unattr		Consp/Heterosp	Mix Consp/mix Heterosp	
% overlap	0.741	0.377		0.023	0.243	
Modal latency	0.501	0.499		0.094	0.194	
Post-Stim. Suppression	0.696	0.486		0.232	0.074	

Attr attractive stimuli, Unattr unattractive stimuli, Consp conspecific stimuli, Heterosp heterospecific stimuli

H. cinerea: being perceived as the lagging call relative to an attractive low-frequency one reduces a male's attractiveness much more than lagging behind an unattractive high-frequency one (Höbel 2010).

Males also exhibited selective attention during the mixed treatment of the interspecific experiment, avoiding call overlap more strongly with the conspecific than the heterospecific stimulus when presented simultaneously. This, too, is consistent with a prior study looking at the importance of cross-species call overlap on female *H. cinerea* preferences: they 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.

Local calling plasticity and chorus-wide effects

Behavioral interaction in chorusing animals are generally limited to small local scales (within a few nearest neighbors); yet, they can give rise to collective phenomena such as overall chorus structure (Greenfield 1994a, b). To stay competitive on a local scale, male *H. cinerea* show selective attention and adjust call features as well as call timing. Local chorus composition, and the changes in calling behavior it generates, may thus affect the overall acoustic environment in the chorus.

In single-species choruses, selective attention and call timing adjustments focused on attractive rivals predict that choruses with a high proportion of attractive males should have overall lower call periods, resulting in a chorus structure that appears acoustically less dense. By contrast, mixed-species choruses, due to the lower proportion of males that require call timing adjustments (selective attention has the males focus on conspecifics), should show overall shorter call periods, and thus appear acoustically more dense. Similar predictions can be made for nightly variation in acoustic density. Call periods should be shortest around the mid-time of nightly chorus activity. This is because initially many males, including very attractive ones that force their neighbors to lower call period to achieve precise call timing adjustments, are present. But as these attractive males enter amplexus with a female, they remove themselves from the chorus and release their neighbors from the need to adjust call timing, thus allowing call periods to become shorter.

Comparison of plasticity in call feature and call timing behaviors in relation to chorus density and/or the presence or absence of heterospecifics may also provide insight about the strength of selection on a particular type of plasticity throughout the breeding season. For example, there may be times with lower chorus density—such as during periods of unfavorable weather conditions or towards the end of the breeding season—in which the reduction of intrasexual competition may result in weaker selection on call timing abilities yet

stronger selection on the ability to maximize the temporal attractiveness of the call itself. This scenario may allow for variation in call timing ability to persist, while simultaneously driving further evolution of plasticity in call features.

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Data availability The datasets generated during this current study are available in the Dryad Digital Repository, <https://doi.org/10.5061/dryad.1555n1k>

Compliance with ethical standards

Ethical approval All experimental procedures were approved by the Animal Care and Use Committee of the University of Wisconsin-Milwaukee (IACUC 07-08#38). All applicable international, national, and/or institutional guidelines for the use of animals were followed.

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