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Interaction between signal timing and signal feature preferences: causes and implications for sexual selection

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Females often choose their mates based on features of the male's advertisement signal, often preferring louder, deeper, longer, or faster signals. Females of chorusing animals also frequently have preferences for signal timing positions, generally preferring the leading signal. Under natural chorus conditions, females must choose among signals that vary in features as well as relative timing. I conducted playback experiments with female *Hyla cinerea* treefrogs to evaluate whether one type of preference (for attractive signal feature or attractive timing) dominates the overall attractiveness of a signal, or whether different types of preferences can interact. I found that the overall attractiveness of a signal was determined by a combination of timing and feature preferences. The specific signal feature (amplitude, frequency, duration, or rate) was important for the outcome of this interaction effect; signal frequency preferences were very strong and dominated overall signal attractiveness, while signal amplitude, duration or rate preferences were weaker and overall signal attractiveness was largely determined by signal timing preferences. I suggest that preference interactions can result in changes in the dynamics of sexual selection and in the maintenance of variation in signal traits, because males with unattractive signals could gain higher mating success by producing these signals in a leading position, thus weakening or even disrupting feature-based signal selection.

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In many species males aggregate during the breeding season and form choruses or leks (Höglund & Alatalo 1995; Gerhardt & Huber 2002; Greenfield 2002). In such cases, mate-searching females are faced with signalling environments characterized by some level of overlap between the displays of different males. Under these conditions, two types of mate preference come into play: preferences for signal features and preferences for the relative timing of signals. Signal feature preferences are those based on particular parameters of the advertisement signal, for example, preferences for signal duration, rate, frequency, colour or amplitude (reviewed in Gerhardt & Huber 2002; Searcy & Nowicki 2005). Signal timing preferences are those based on the relative timing of the signals of different males (Gerhardt & Huber 2002; Greenfield 2002). Signal timing preferences occur in a wide range of taxa and signal modalities (reviewed in: Gerhardt & Huber 2002; Greenfield 2002; Reaney et al. 2008). It is generally the leading of two signals in close succession that is preferred (Whitney & Krebs 1975; Dyson & Passmore 1988a; Klump & Gerhardt 1992; Grafe

1996; Greenfield et al. 1997; Bosch & Márquez 2002; but see Grafe 1999).

Little is known about the potential interaction between signal feature and signal timing preferences. This is a problem because mate choice often takes place under conditions in which females have to choose between signals that vary in acoustic features as well as in relative timing. A female may encounter situations in which a signal with unattractive features appears in an attractive temporal position, and vice versa. In such cases, there may be interactions between preferences of different types, such that the signal that is most attractive may not necessarily be predictable on the basis of a single type of preference. For example, signal feature preferences may be moderated or even dominated by signal timing preferences, such that males with otherwise unattractive signals may gain higher mating success by producing signals in a leading position. Such interactions have therefore the potential to change the dynamics of sexual selection.

Studies looking for an interaction between signal feature and signal timing preferences have been conducted with few species, but include examples from insects and anurans. This suggests that preference interactions are taxonomically widespread, and thus of potentially great importance for signal selection in group-displaying species in general. So far, interactions between signal

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timing preferences and preference for the features amplitude and frequency have been described. The general pattern in anurans and katydid, two groups that have been the focus for studies of sexual selection and communication, seems to be that preferences based on signal amplitude are dominated by signal timing preferences. An amplitude difference of more than 6 dB (that is, twice the signal energy) in favour of the lagging signal is necessary to recapture the feature preference (Dyson & Passmore 1988b; Klump & Gerhardt 1992; Grafe 1996; Snedden & Greenfield 1998; Römer et al. 2002; Fertschai et al. 2007). Signal timing preferences also dominate or at least equalize preferences based on signal frequency in the few anurans tested so far (Dyson & Passmore 1988a; Howard & Palmer 1995; Grafe 1996). Little is known about the effect of signal timing preferences on signal rate preferences (but see Berg & Greenfield 2005), and signal duration preferences have to my knowledge never been examined in this respect. Signal duration preferences are especially problematic because examples of preferences for longer or more frequently repeated signals are particularly prevalent in nature (Ryan & Keddy-Hector 1992; Greenfield 2002), and finding that these feature preferences are affected by signal timing effects and preference interactions could change the expectations for the direction and strength of selection on signals.

The green treefrog, *Hyla cinerea*, presents an ideal opportunity to study the challenges faced by organisms that display and choose mates in dense choruses, where they are confronted with multiple, overlapping signals varying in signal features (e.g. frequency, intensity, duration and repetition rate). I evaluated whether signal timing and signal feature preferences interact with each other, and, if so, what determines overall signal attractiveness. To do this I conducted playback trials with *H. cinerea* females in which I simultaneously varied both signal timing as well as signal features.

First, I evaluated whether different types of preference (feature or timing) interact with each other. If signal feature preferences are key for overall signal attractiveness, then a signal with attractive features should always be preferred, independent of its relative timing. If, on the other hand, signal timing preferences are key for overall attractiveness, then a leading signal should always be preferred, independent of its signal features. If preferences interact, then the attractiveness of a signal feature should vary as a function of the relative timing in which it is presented. For example, a signal that is preferred during alternating signal presentation may become unattractive if it is presented in a lagging position.

If preferences interact, there may be different causes for the interaction. For example, a usually attractive signal with preferred features might lose its attractiveness when it is presented in a lagging position because the signal timing preference (favouring leading signals) dominates the interaction. In this scenario overall signal attractiveness would be determined by the stronger preference (preference strength hypothesis). A signal with normally preferred features might also lose its attractiveness if females become unable to exert signal feature preferences when signals overlap (preference impairment hypothesis). In this scenario, signal overlap, particularly high degrees of signal overlap, impairs a female's ability to perform phonotaxis to the source of a preferred signal. If females have difficulty performing phonotaxis, this should be reflected in the amount of time they take to reach the speaker broadcasting a preferred signal, and the effect should increase as the degree of signal overlap increases. Likewise, difficulties in performing phonotaxis should also be reflected in the frequency at which females reverse course during phonotaxis (i.e. start to walk towards one speaker, then turn around and ultimately approach the other speaker), and the effect should increase as the degree of signal overlap increases.

METHODS

Study Species

The green treefrog is a common inhabitant of lakes, ponds and swamps in the southeastern United States (Conant & Collins 1998). During spring and summer, males aggregate to advertise for females. Size and density of choruses can vary widely, from a few males spaced far apart to large, dense choruses with hundreds of calling males. Males start calling activity shortly after dark, and continue to signal for 2–4 h each night. The advertisement signal consists of a single pulse, lasting on average 163 ms, which is repeated every 738 ms, and there is considerable geographical variation in these temporal signal parameters (Asquith et al. 1988). The communication system of *H. cinerea* is well studied. Females show preferences for a range of signal features; they prefer average frequency over high-frequency signals (Gerhardt 1974; Höbel & Gerhardt 2003), louder signals, longer signals, and signals produced at higher rates (Gerhardt 1987). Females also show signal timing preferences. If two signals overlap, they prefer the leading of the two signals (Klump & Gerhardt 1992; Höbel & Gerhardt 2007). But as soon as there is a short silent interval between consecutive signals, relative signal timing no longer influences female choice (Höbel & Gerhardt 2007).

Study Site

I conducted trials at Private John Allen National Fish Hatchery, Lee Co., Mississippi, U.S.A., during the field seasons (May–July) of 2001, 2003 and 2005. The average \pm SD temperature on nights that testing took place was 20.5 ± 2.4 °C. Temperature did not vary significantly between years ($F_{2,23} = 2.19$, $P = 0.14$). I collected pairs in amplexus to ensure that all tested females would be sexually responsive. All females were tested only once with a given signal pair, and there was a time-out period of at least 5 min between different tests. Within tests of a series of females with the same pair of stimuli, I alternated the stimuli between loudspeakers to guard against side biases. I made all behavioural observations under dim red light and released all females at the site of capture within 1 day of being tested. Experimental procedures adhered to the ASAB/ABS Guidelines for the Use of Animals in Research, and were approved by the Animal Care and Use Committee of the University of Missouri (ACUC protocol no. 1910).

Playback System

I tested all females in a two-choice playback design in a portable playback arena set up in the field. The playback arena was 2 m long and 1 m wide. The floor of the arena was a plywood board, the sides 50 cm high wood frames screened with lightweight black cloth. The speakers (RadioShack Optimus XTS-40) were placed 2 m apart, facing each other along the central long axis and just outside the arena. Sound files were broadcast from a laptop computer at a sampling rate of 44.1 kHz, using CoolEdit96 software (Syntrillium Software, Scottsdale, AZ, U.S.A.). The frequency response of the system was flat within ± 2 dB from 0.4 to 4 kHz. Unless otherwise indicated, the sound pressure level (SPL in dB re. 20 μ Pa, fast root-mean-square, RMS) of each signal was equalized to 85 dB using a Lutron SL-4001 sound level meter prior to each test.

For testing, I placed females individually in a small round wire cage (10 cm diameter) midway between the loudspeakers. Once the alternative stimuli had been played back for at least five repetitions, I remotely removed the lid of the release box by pulling a string, so that the female could move freely. I scored a positive response once a female touched the cloth in front of the speaker.

I used a stopwatch (Extech Instruments 365510) to time (to the nearest second) each female's approach from the moment I lifted the lid of the release cage until the female reached the speaker broadcasting the preferred signal (=approach time). I also sketched the approach path that each female took while moving inside the playback arena. These sketches allowed me to later determine whether the female approached a chosen speaker directly, or whether she started walking towards one speaker, but reversed course and choose the other speaker (=approach course).

Experimental Design

To test for female preferences I generated synthetic stimuli modelled after the advertisement signal of *H. cinerea*. Stimuli were generated from 16-bit digital files created by custom-designed software (courtesy of J. Schwartz). Unless otherwise indicated, the stimuli had three frequency components (0.9 + 2.7 + 3.0 kHz), a duration of 150 ms, a rise time of 25 ms (inverse exponential), and a fall time of 50 ms (inverse exponential). A signal with these values is close to the average values of signal frequency and signal duration of the species, and is hereafter referred to as the 'standard signal' (Gerhardt 1987). For trials testing for the interaction of signal timing with signal frequency and signal duration preferences, I generated modified versions of this standard signal by manipulating signal frequency or duration (see below).

To test for an interaction of signal timing preference and the signal feature preferences for amplitude, frequency and duration, I created stereo files that played an unattractive signal on the left channel and an attractive signal on the right channel. For each experiment I varied only one signal feature in the presented alternatives and set the other features near the average values for the population (85 dB SPL, 0.9 kHz, 150 ms duration, 800 ms signal period, respectively). I presented the alternatives in six relative timing positions: 100% overlap, 83% overlap, 50% overlap, abutting, outside forbidden interval, and alternating. Earlier experiments with identical alternatives revealed that a signal presented 25 ms after the offset of the leading alternative no longer showed reduced attractiveness, hence this signal timing position was called 'outside forbidden interval' (Höbel & Gerhardt 2007). In trials in which alternatives were presented in a leader–follower relationship, I arranged them so that the alternative with the unattractive signal feature value was leading the attractive one (see cartoons in Figs 1, 3, 5).

To investigate the interaction of signal timing and signal amplitude preferences, I conducted two experiments. In experiment 1 of the signal amplitude trials, I set the attractive unattenuated signal to 85 dB SPL and the unattractive attenuated signal to 79 dB SPL (i.e. a 6 dB difference). In experiment 2 of the signal amplitude trials, I kept the relative timing of the alternatives constant (at 100% overlap, 50% overlap, or alternating, respectively), but varied the attenuation levels to a greater degree than in experiment 1 (in 3 dB steps from 0 to 15 dB attenuation difference; see Results, Fig. 2). In the signal frequency trials, the attractive alternative was a signal of average frequency (0.9 + 2.7 + 3.0 kHz) and the unattractive alternative was a signal of higher frequency (1.1 + 3.6 + 3.9 kHz). Higher frequencies are representative of smaller conspecific males and are less attractive to females than average- or lower-frequency signals (0.9 kHz and below; Höbel & Gerhardt 2003). Based on the regression of signal frequency on size (snout–vent length in mm) and on weight (in mg) of males from the study population, the difference of 0.3 kHz is equivalent to a size difference of about 7% and a weight difference of about 31% (unpublished data). In the signal duration trials, the attractive alternative was a longer signal of 180 ms duration and the unattractive one was a shorter signal of 140 ms duration.

Finally, to test for an interaction between signal timing preference and signal feature preference for repetition rate, I gave females a choice between a slower and a faster signal rate. One channel always played the signals at a rate of 75 signals/min, which is close to the average rate in this population (78 signals/min; unpublished data). I conducted two experiments. In experiment 1 of the signal rate trials, I tested the average signal rate versus half the rate (38 signals/min). In experiment 2 of the signal rate trials, I tested the average rate versus the double rate (150 signals/min; see Results, Fig. 6a, b). In each case I presented the alternatives in two relative timing positions: in one, I arranged the alternatives in alternating position; in the other, I arranged the alternatives so that the slower one was always presented in leading position. Since the faster alternatives contained twice as many signals (see above), this resulted in the signals in the faster alternatives being presented as one overlapped, lagging signal followed by one signal that was free of signal overlap.

In each of the experiments I obtained data to address both the question of whether preference types interact, as well as data on whether overall signal attractiveness is due to preference strength or preference impairment. To evaluate whether preferences interact, and to test the preference strength hypothesis, I compared the proportions of females approaching each of the tested feature/timing combinations. To evaluate the preference impairment hypothesis, I used two approaches: (1) I compared the approach times of females during each of the tested feature/timing combinations; (2) I compared the approach course of females, by looking at the proportion of females that reversed course during phonotaxis to each of the tested feature/timing combinations.

Statistical Analysis

I attempted to test at least 10 females with each combination of stimuli, and tested additional females whenever possible. I report the female preference results as the proportions of females that chose one of the alternatives (Figs 1–3, 5, 6), and compute confidence limits from standard tables (Burstein 1971). I base the confidence limits on confidence levels of $\gamma_1 = 95\%$, and interpret the confidence limits as Bayesian credible intervals, which correspond numerically to confidence limits when the prior distribution is uniform (see Gerhardt 1992; Gerhardt et al. 2007). Here, the criterion for judging that a preference is significant is when the upper confidence limit is below 0.4 (rejection of alternative) or the lower confidence limit is above 0.6 (preference for alternative) (see Gerhardt 1992; Gerhardt et al. 2007; Höbel & Gerhardt 2007). For comparison, I also provide *P* values from two-tailed binomial tests, and indicate a significant difference ($P < 0.05$) for an alternative, or significant discrimination against an alternative, by an asterisk adjacent to the test's symbol in the respective figure. However, these *P* values are not strictly valid, because sample sizes were not fixed ahead of time (see Gerhardt 1992).

The preference strength hypothesis predicts that overall signal attractiveness is determined by the stronger preference (either for signal feature or timing). To test this prediction I compared whether the proportion of females choosing a signal (e.g. higher-frequency signal) changed with its relative timing. If the proportion of females choosing the signal remains constant irrespective of signal timing, this would indicate that the signal feature preference is the stronger preference. If the proportion of females preferring the signal is higher when it is in leading position, this would indicate that the signal timing preference is stronger.

The preference impairment hypothesis predicts that, as signal overlap increases, (1) the time it takes females to reach the signal source will increase, and (2) the proportion of females that reverse course during phonotaxis will increase. Because females may

approach attractive signals faster or more directly than unattractive ones, I incorporated the female's chosen signal into the analysis. I used a model that included relative signal timing and the female's chosen signal (e.g. shorter or longer in the case of duration trials) as main terms, as well as their interaction, to test for differences in approach time and approach course as a function of signal overlap and chosen signal. The model included linear and quadratic terms for signal timing. For the purpose of these tests, signal timing is best viewed as a continuous variable, so I used signal delay values corresponding to different degrees of signal overlap to code for signal timing. Thus, a signal delay of 0 ms is equivalent to 100% overlap, a delay of 25 ms to 83% overlap, a delay of 75 ms to 50% overlap, a delay of 150 ms to a signal in abutting position, a delay of 200 ms to a signal that is outside the forbidden interval of female preferences, and a delay of 400 ms to signals exactly alternating with each other. I log-transformed signal delay values (coding 0 ms delay as 1), and I arcsine-transformed the data for the proportion of females changing approach directionality. I conducted all statistical tests with JMP 7.0.1 (SAS Institute, Cary, NC, U.S.A.).

RESULTS

I tested 93 frogs for this experiment ($N = 30$ in 2001, $N = 34$ in 2003, $N = 29$ in 2005). The average \pm SD number of signal combinations tested per female was 4.8 ± 3.6 (range 1–19; mode 3). Sample sizes for each tested signal combination are provided in the corresponding figures (Figs 1–3, 5, 6).

Signal Amplitude

Signal attractiveness was determined by an interaction between timing and amplitude preferences (Figs 1, 2). In experiment 1 of the signal amplitude trials, the amplitude difference between attractive and unattractive stimuli was 6 dB and the relative timing of these stimuli was modified (Fig. 1). Depending on relative timing, females either preferred the unattenuated alternative, showed no

preference for either alternative, or preferred the attenuated alternative. In experiment 2 of the signal amplitude trials, the relative timing of the alternatives was kept constant (at 100% overlap, 50% overlap or alternation) and the amplitude difference was modified (Fig. 2). When signals were presented in 100% overlap, females did not discriminate between alternatives of the same amplitude (0 dB attenuation), but always preferred the unattenuated alternative (Fig. 2a). With 50% overlap, signal timing preferences overrode amplitude preferences for differences of up to 6 dB, while both preference types were equalized at 9 dB differences; at larger amplitude differences (>12 dB) females preferred the lagging (unattenuated) alternative (Fig. 2b). When signals were presented in alternation, females did not discriminate between signals of the same amplitude (0 dB attenuation), but tended to prefer the unattenuated alternatives (Fig. 2c). The results of both experiments suggest that signal timing preferences are stronger than amplitude preferences, and support the preference strength hypothesis.

To test the preference impairment hypothesis I used data only from experiment 1 of the signal amplitude trials (Fig. 1). The time it took females to approach their chosen signal was not influenced by signal overlap, the particular signal that was chosen, or their interaction (Table 1). Overall, mean \pm SD approach time in the amplitude experiment was 76 ± 58 s. In 11% of these trials, the female started walking towards one alternative, then reversed course and chose the other alternative. The frequency of these changes in course was not affected by signal overlap or the particular signal that was chosen (see Table 2). These results reject the preference impairment hypothesis.

Signal Frequency

Signal attractiveness was determined by an interaction between timing and frequency preferences. Depending on relative timing, females either preferred the attractive low-frequency signal or did not discriminate between the alternatives (Fig. 3). However, the

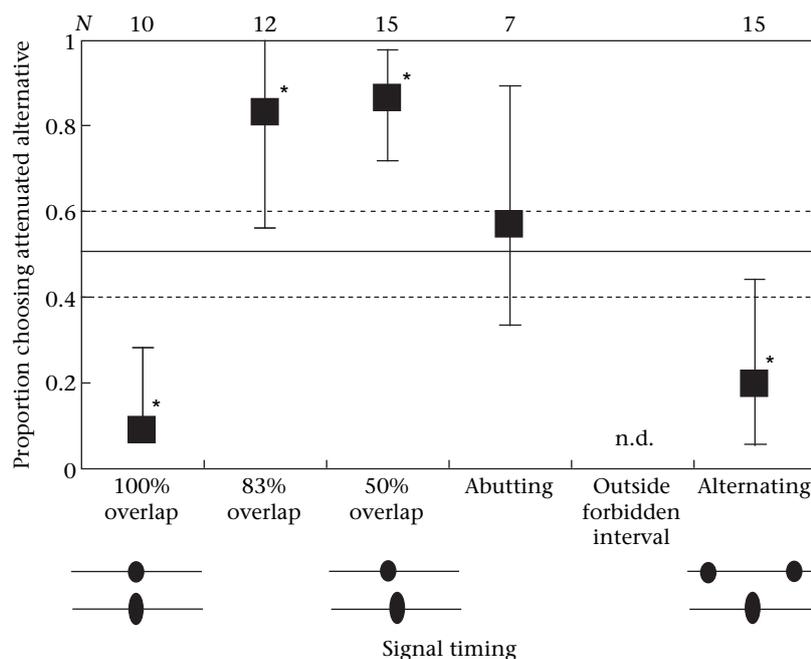


Figure 1. Proportions of female green treefrogs choosing the attenuated alternative at different relative signal timing positions. Relative amplitude difference was kept constant at 6 dB. Numbers above symbols show the sample size; n.d.: no data. Asterisks indicate a significant difference ($P < 0.05$) for an alternative, or significant discrimination against an alternative.

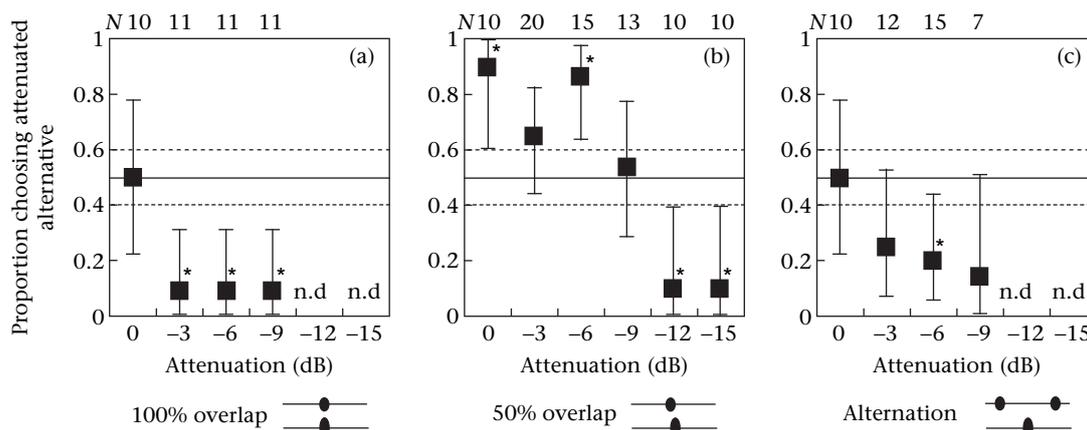


Figure 2. Proportions of female green treefrogs choosing the attenuated alternative at different relative signal timing positions: (a) 100% overlap, (b) 50% overlap and (c) alternation. Relative timing of the two alternatives was kept constant, but the relative amplitude of the alternatives was modified. Numbers above symbols show the sample size; n.d.: no data. Asterisks indicate a significant difference ($P < 0.05$) for an alternative, or significant discrimination against an alternative.

frequency preference was never reversed (i.e. even in leading position, females never significantly preferred the unattractive high-frequency signal). This finding suggests that frequency preferences are stronger than signal timing preferences, and support the preference strength hypothesis.

There was no relationship between the degree of signal overlap, the individual female's chosen signal and the time that it took females to approach a speaker (Table 1). Average \pm SD approach time was 72 ± 43 s.

In 16% of the frequency trials a female started walking towards one alternative, then reversed course and chose the other alternative. The full model (Table 2) showed no effect of timing or chosen signal on approach course. Removing the nonsignificant quadratic term to improve power revealed a significant effect of

timing ($F_{1,7} = 6.052, P = 0.044$): there were more course reversals at higher degrees of overlap. This finding appears consistent with the impaired preferences hypothesis. In this reduced model, the magnitude of the F ratio for the interaction between timing and chosen signal ($F_{1,7} = 2.079, P = 0.19$) suggests that its lack of significance may be due to low statistical power, rather than a small effect. I thus tested for a relationship between relative timing and the frequency of course reversal separately for each type of chosen signal, focusing on the magnitude of the slopes rather than their significance, since the small sample sizes for each case ($N = 6$) afford little statistical power. For females that chose the average-frequency signal, the slope was -0.18 , while for females that chose the high-frequency signal, the slope was -0.69 (see Fig. 4a), indicating that females that eventually chose the unattractive high-frequency signal more often started out by walking towards the attractive average-frequency signal, but then reversed course. Thus, rather than an impaired exercise of preferences, I interpret this finding as a change in the preference that dominates: for some females, the frequency preference was initially stronger, but was then superseded by the timing preference.

In short, the results of the approach time analysis do not support the preference impairment hypothesis. The results for the approach

Table 1
Effect of signal timing (overlap to alternation) and chosen signal (signal feature) on approach time of female green treefrogs

Source	df	F	P
Signal amplitude			
Timing	1, 56	0.339	0.563
Timing×timing	1, 56	1.549	0.219
Chosen signal	1, 56	0.001	0.980
Timing×chosen signal	1, 56	0.273	0.603
Timing×timing×chosen signal	1, 56	1.666	0.202
Signal frequency			
Timing	1, 62	0.544	0.463
Timing×timing	1, 62	0.033	0.587
Chosen signal	1, 62	0.200	0.656
Timing×chosen signal	1, 62	0.087	0.769
Timing×timing×chosen signal	1, 62	0.226	0.634
Signal duration			
Timing	1, 69	0.000	0.991
Timing×timing	1, 69	0.996	0.322
Chosen signal	1, 69	1.868	0.176
Timing×chosen signal	1, 69	0.023	0.880
Timing×timing×chosen signal	1, 69	0.091	0.764
Signal rate (set 1)			
Timing	1, 20	0.118	0.735
Chosen signal	1, 20	1.212	0.284
Timing×chosen signal	1, 20	0.319	0.588
Signal rate (set 2)			
Timing	1, 21	1.588	0.222
Chosen signal	1, 21	0.019	0.893
Timing×chosen signal	1, 21	0.393	0.537

Table 2
Effect of signal timing (overlap to alternation) and chosen signal (signal feature) on approach course of female green treefrogs

Source	df	F	P
Signal amplitude			
Timing	1, 4	0.005	0.948
Timing×timing	1, 4	2.554	0.281
Chosen signal	1, 4	0.345	0.578
Timing×chosen signal	1, 4	0.356	0.583
Timing×timing×chosen signal	1, 4	0.051	0.833
Signal frequency			
Timing	1, 5	3.796	0.109
Timing×timing	1, 5	0.444	0.535
Chosen signal	1, 5	0.0003	0.988
Timing×chosen signal	1, 5	1.519	0.273
Timing×timing×chosen signal	1, 5	0.299	0.608
Signal duration			
Timing	1, 5	6.730	0.049
Timing×timing	1, 5	2.720	0.160
Chosen signal	1, 5	0.004	0.952
Timing×chosen signal	1, 5	0.741	0.429
Timing×timing×chosen signal	1, 5	0.088	0.779

Significant P values are shown in bold.

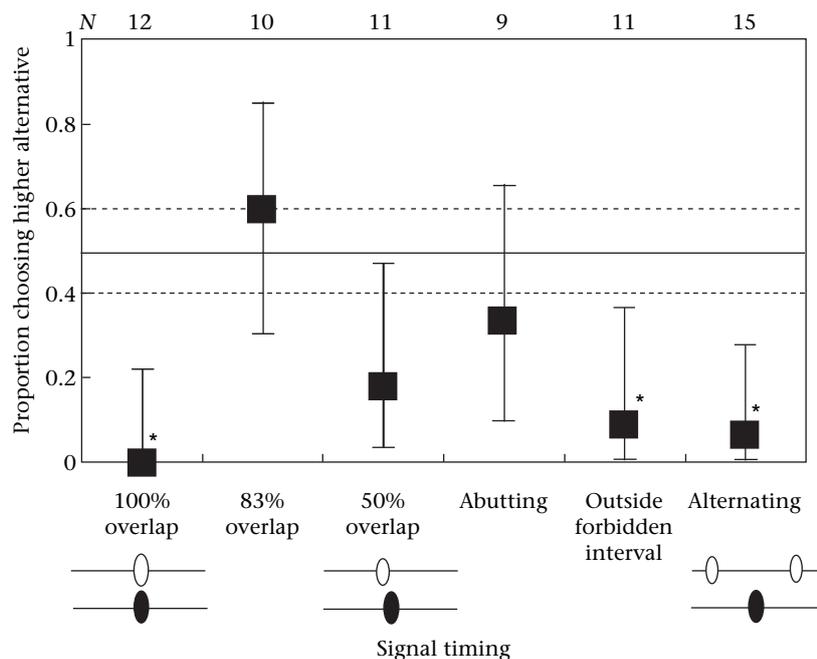


Figure 3. Proportions of female green treefrogs choosing the high-frequency alternative at different relative signal timing positions. Numbers above symbols show the sample size. Asterisks indicate a significant difference ($P < 0.05$) for an alternative, or significant discrimination against an alternative.

course analysis (i.e. significant effect of timing) are consistent with the preference impairment hypothesis, but when considering the interactions between timing \times chosen signal, the results are also consistent with the explanation that initial feature preferences (here for the attractive, average-frequency signal) can be taken over by timing preferences (here for the leading signal).

Signal Duration

Signal attractiveness was determined by an interaction between signal timing and signal duration preferences. Depending on relative timing, females either preferred the attractive longer alternative, showed no preferences for either alternative, or preferred the unattractive shorter alternative (Fig. 5). Preferences for the shorter alternative occurred only when that signal was in leading position. This finding suggests that timing preferences are stronger than signal duration preferences, and support the preference strength hypothesis.

There was no relationship between the degree of signal overlap, the individual female's chosen signal and the time that females took to approach a speaker (Table 1). Average \pm SD approach time was 90 ± 57 s.

In 15% of the duration trials a female started walking towards one alternative and then reversed her approach and chose the other alternative. The full model (Table 2) showed a significant effect of timing on approach course. Removing the nonsignificant quadratic term to improve power further revealed that there might be an interaction between timing and chosen signal. In this reduced model, the magnitude of the F ratio for the interaction between timing and chosen signal ($F_{1,7} = 1.756$, $P = 0.227$) suggests that its lack of significance may be due to low statistical power, rather to a small effect. I thus tested for a relationship between relative timing and the frequency of course reversals separately for each type of chosen signal, focusing on the magnitude of the slopes rather than their significance, since the small sample sizes for each case ($N = 6$) afford little statistical power. For females that chose the attractive long signal, the slope was 0.076, while for females

that chose the short signal, the slope was 0.426 (Fig. 4b), indicating that females that eventually chose the unattractive short signal more often started out by walking towards the attractive long signal but then reversed course. Importantly, the slopes were positive; that is, more females reversed course when there was less signal overlap. In short, the results of the approach time analysis do not support the preference impairment hypothesis, and the approach course analysis in fact provides evidence against this hypothesis.

Signal Rate

Signal attractiveness was affected by an interaction between timing and signal rate preferences. A higher proportion of females chose the alternatives that had signals positioned in a leading position, despite lower signal rate (Fig. 6a, b, left data points). This pattern was more apparent in experiment 2 (Fig. 6a) but was not statistically significant.

At first glance, the control trials (alternation) yielded somewhat unexpected results, because females at the Mississippi study site did not prefer the higher signal rate alternatives (Fig. 6a, b, right data points). This result contrasts with findings of rate preferences in frogs in general (review: Gerhardt & Huber 2002) and with results of two published studies with *H. cinerea* (Gerhardt 1987; Humfeld 2008; from sites in Georgia and Missouri, U.S.A., respectively). However, female *H. cinerea* show geographical variation in other call feature preferences (Höbel & Gerhardt 2003), and preferences for high signal rates are absent or reduced in other populations as well (unpublished data).

Average \pm SD approach time was 78 ± 31 s for experiment 1 of the signal rate trials (slow/average rate) and 76 ± 58 s for experiment 2 of the signal rate trials (average/fast rate) (Table 1).

In 17% of trials in experiment 1 and in 0% of trials in experiment 2 of the signal rate trials, a female started walking towards one alternative, then reversed course and chose the other alternative. Because in these trials only two timing values (overlapping and alternating) and two chosen signal values were available, they

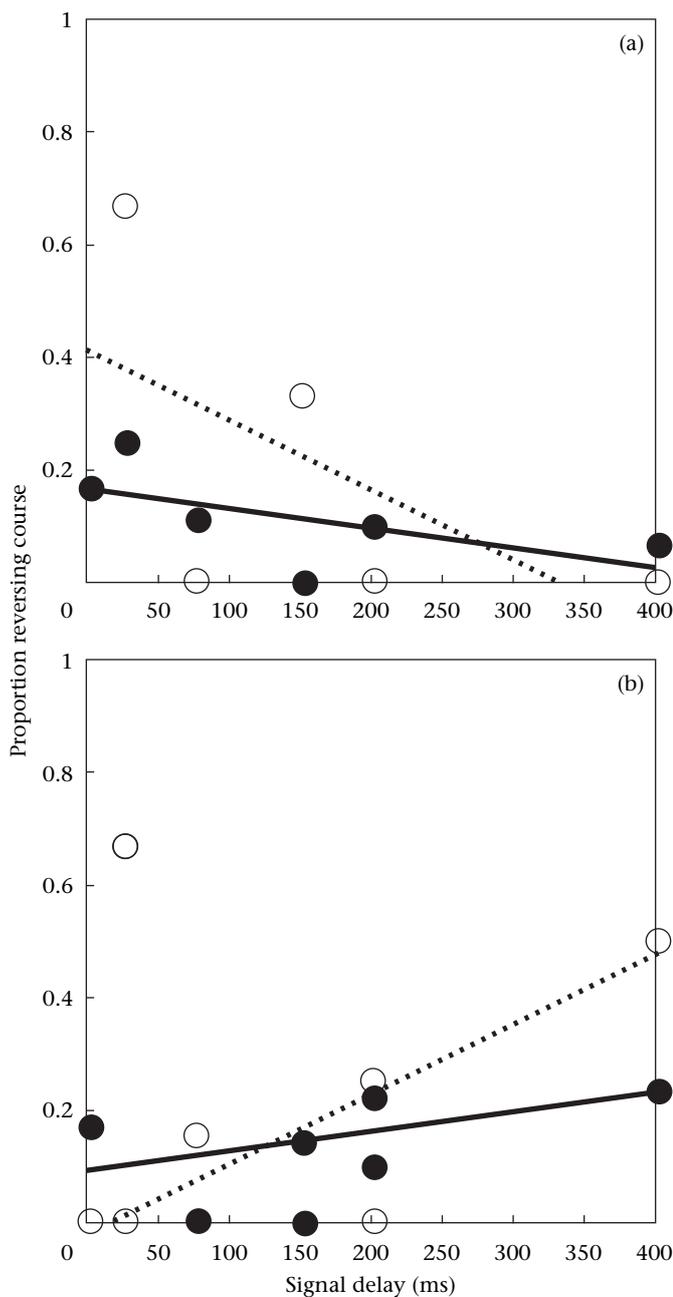


Figure 4. Regression analysis of the effect of signal timing on the proportion of female green treefrogs that reversed course during phonotaxis, as a function of the chosen signal (using untransformed data). (a) Signal frequency trials: regression slopes differed for females that chose the attractive average-frequency signal (filled symbols, solid line) and those that chose the unattractive high-frequency signal (open symbols, dashed line). (b) Signal duration trials: regression slopes differed for females that chose the attractive long signal (filled symbols, solid line) and those that chose the unattractive short signal (open symbols, dashed line). Signal delay values corresponding to different degrees of signal overlap were used to code for signal timing (signal delay of 0 ms = 100% overlap, 25 ms = 83% overlap, 75 ms = 50% overlap, 150 ms = abutting, 200 ms = outside the forbidden interval, 400 ms = alternation).

could not be analysed with the statistical models used above. I therefore used chi-square tests, with separate analyses for females that chose the slow signal and those that chose the average signal in experiment 1 of the signal rate trials. Regardless of whether females chose the slow or the average signal, they did not reverse courses more often when signals overlapped than when they

alternated (in both tests: $P > 0.5$). It was not possible to analyse approach course for the second experiment of the signal rate trials, because none of the females reversed course during phonotaxis. In summary, both the results of the approach time and the approach course analyses do not support the preference impairment hypothesis.

DISCUSSION

Females choosing a mate under natural conditions are often faced with communication environments in which signals vary in features as well as relative timing. In *H. cinerea* treefrogs, preferences interact, and the overall attractiveness of a signal is generally determined by a combination of timing and feature preferences.

I evaluated two hypotheses about the causes of this preference interaction. The preference strength hypothesis predicts that overall signal attractiveness is determined by the stronger preference (either for signal feature or timing). The data support this hypothesis. Sometimes overall signal attractiveness was largely determined by the signal feature preference (see Fig. 3) and sometimes the signal timing preference had a larger influence on overall signal attractiveness (Figs 1, 5). Interestingly, I did not find a generalized dominance of signal feature over signal timing preferences. Rather, the specific signal feature preference in question (for amplitude, frequency, duration or rate) varied in strength relative to the signal timing preferences; signal frequency preferences were very strong and heavily dominated overall signal attractiveness, while signal amplitude, duration or rate preferences seemed comparatively weaker, and overall signal attractiveness was largely dominated by signal timing preferences.

I found no support for the preference impairment hypothesis. This hypothesis predicts that signal overlap impairs a female's ability to perform phonotaxis to the source of a preferred signal, and that this effect should increase as signal overlap increases. I tested this hypothesis in two ways. First, I analysed whether females took longer to reach the source of a preferred signal (approach time) when signal overlap was higher. I found that approach times did not vary as a function of signal timing (Table 1), suggesting that female green treefrogs did not have problems with phonotaxis, even at high levels of signal overlap. Second, I analysed whether females reversed course more often when signal overlap was higher. Again, I found little support for this hypothesis. The results of the signal duration trials even provided support against this hypothesis, since females reversed course more frequently when signals did not overlap (Fig. 4b). Similar results have been obtained from females of other frog species (Passmore & Telford 1981; Backwell & Passmore 1991), but there are also examples where signal overlap does affect accurate phonotaxis (Marshall et al. 2006). Future research should evaluate whether the fine-temporal structure of a species' signal is related to the severity with which signal overlap impacts phonotaxis. Species with pulsed signal structure (Marshall et al. 2006) may be more impaired by signal overlap than those with tonal signal structure (Passmore & Telford 1981; this study).

Chorus density may have a profound influence on the outcome of interactions between signal timing and signal feature preferences documented in this (Figs 1–6) and other studies (Dyson & Passmore 1988a, b; Grafe 1996; Snedden & Greenfield 1998; Berg & Greenfield 2005). Although males are capable of precise signal timing relative to signals of their neighbours (reviewed in Greenfield 2005), the degree of signal overlap in natural choruses will be strongly influenced by local densities of displaying males (Schwartz et al. 2002). This is due to the mechanism that males use to adjust the timing of their signals relative to those of other males. Signal timing in *H. cinerea* is mediated via an inhibitory-resetting

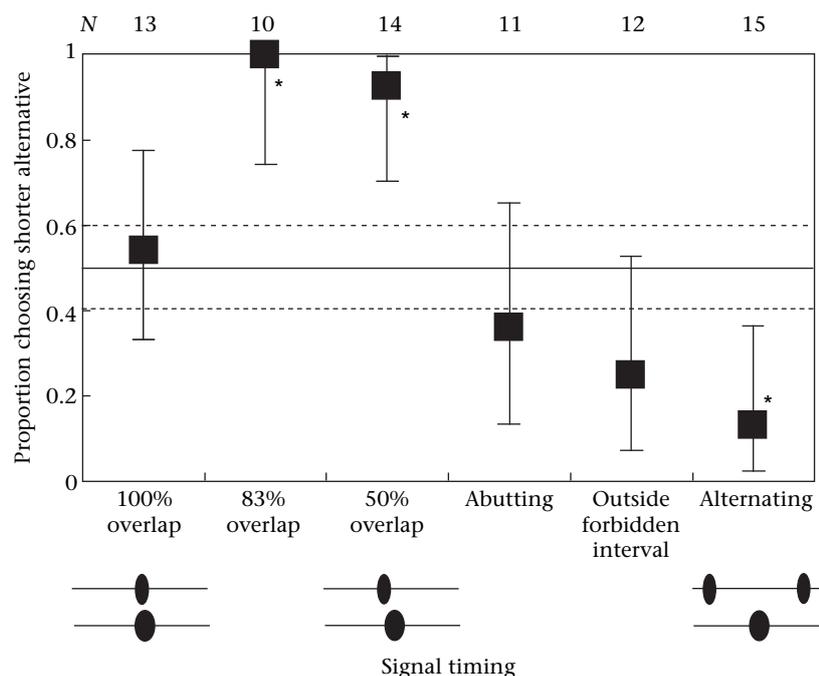


Figure 5. Proportions of female green treefrogs choosing the shorter alternative at different relative signal timing positions. Numbers above symbols show the sample size. Asterisks indicate a significant difference ($P < 0.05$) for an alternative, or significant discrimination against an alternative.

mechanism (Höbel & Gerhardt 2007), where the perception of a rival's signal resets and inhibits the production of a male's signal until after the end of the interfering signal (Greenfield 1994). In addition, frogs also use a mechanism of selective attention by which they selectively apply this inhibitory mechanism to only a subset of two or three of their closest (loudest) neighbours (Greenfield & Rand 2000). In low-density choruses, males are likely to have so few neighbours that they can largely avoid signal overlap. This reduces the chance that preference interactions can occur, resulting in an overall stronger influence of signal feature preferences on signal attractiveness. The expectation for high-density choruses is a higher frequency of signal overlap, particularly with more remote males, a higher frequency of preference interactions, and a stronger influence of signal timing preferences on signal attractiveness.

In short, variation in chorus density has the potential to change the dynamics of sexual selection, changing the focus of sexual selection due to female choice between signal features and signal timing. This suggests that there may be several signal feature/timing combinations that represent different fitness peaks across the signalling landscape. These different fitness peaks may depend on chorus density, such that in low-density choruses, signal features may contribute more to overall signal attractiveness, while in denser choruses, the timing of the signal may become more important.

At a more proximate level, the interaction between preference types suggests that plasticity in the mechanism of inhibitory-resetting used by males to time their signals may be favourable. The degree to which plasticity in signal timing can increase a male's attractiveness will depend on whether the interaction between preference types modifies whether signal features and timing determine signal attractiveness. For example, in *H. cinerea*, some signal feature preferences (such as those for signal rate and signal duration) would allow males with unattractively slow or short signals to increase their attractiveness by achieving leading positions. By contrast, the stronger preference for signal frequency was

robust to signal timing, and thus males with unattractively high-frequency signals (i.e. small males) would never be capable of increasing their attractiveness by achieving leading signal placement.

Whether males show plasticity in signal timing, and are thus capable of modifying their relative attractiveness by adaptive signal timing remains to be investigated. One potential avenue could be through changes in the parameters of the inhibitory-resetting mechanism. For example, a shallower slope of the rebound from inhibition would increase the likelihood that a male's signal falls before the signal of his rival and becomes an attractive leading signal. Another option that could increase the likelihood that a signal is produced before the rival's next signal is by speeding up overall signal production rate. This is suggested by a study by Richardson et al. (2008), which showed that European treefrog, *Hyla arborea*, males that called with higher signal rate also produced more leading signals.

Elucidating effects of preference interactions will yield a more realistic picture of sexual selection by female choice in group-breeding animals, which have been the focus of much research on animal communication and sexual selection. For example, hypotheses about the form of selection on signals are frequently based on the results of playback trials, and males with signal parameters shown to be preferred in those trials are expected to have higher mating success. When scoring mating success in the wild, however, it is frequently found that there is a mismatch between the predicted and realized mating success, and this, in turn, is commonly interpreted as females being unable to execute choice under natural (i.e. more complex) conditions (Gerhardt 1982; Dyson & Passmore 1988a). However, as this and other studies show, females are well capable of executing choice even under more challenging conditions (e.g. Passmore & Telford 1981; Backwell & Passmore 1991). The cause for the mismatch between predicted and realized mating success may instead lie in the type of preference that dominates overall signal attractiveness under the different conditions in which females are making their choices.

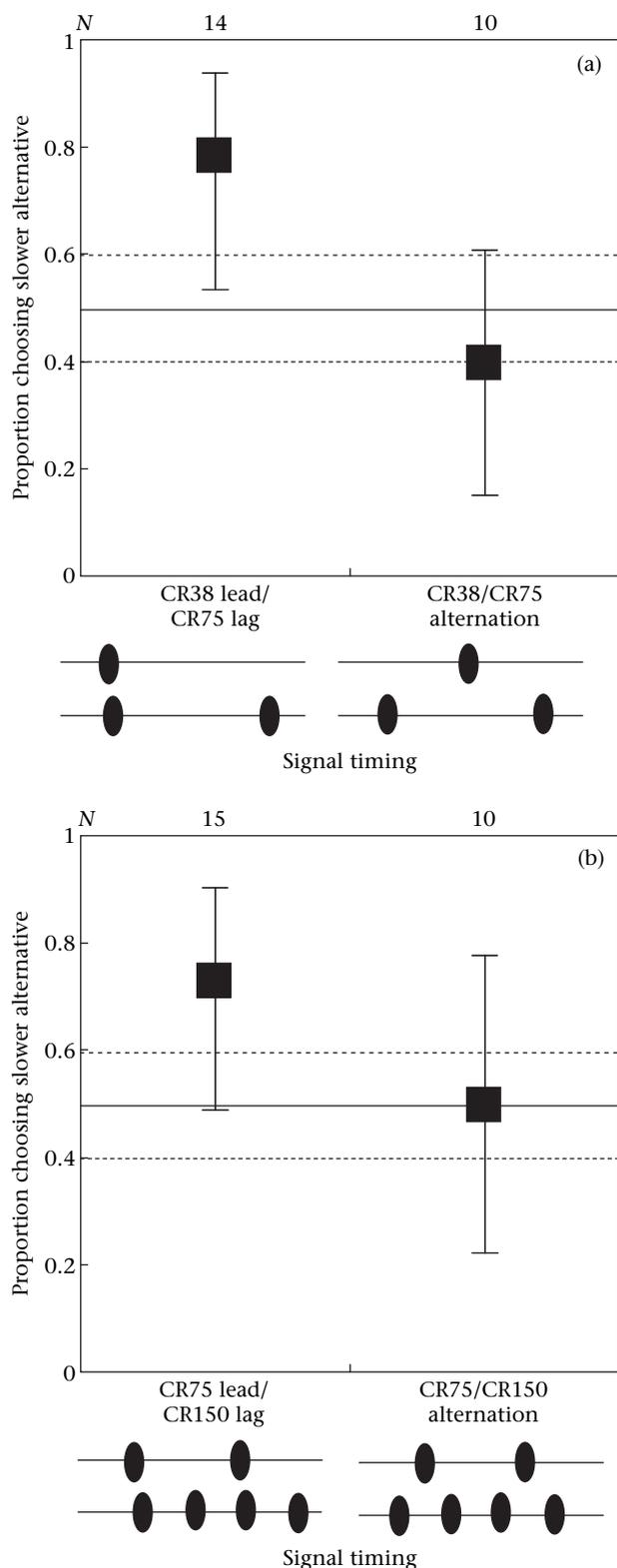


Figure 6. Proportions of female green treefrogs choosing the slow rate alternative at different relative signal timing positions (leading–lagging versus alternating). (a) Testing a slow rate (38 signals/min) against the average rate (75 signals/min). (b) Testing the average rate (75 signals/min) against a fast rate (150 signals/min). Numbers above symbols show the sample size.

Choice trials are similar to low-density (simple) conditions, and here, signal-feature-based preferences may dominate because low levels of background noise and little signal overlap allow for critical evaluation of signal differences. Conditions in natural choruses are often characterized by high-density (complex) situations, and signal timing preferences may dominate because picking out the leading signal may be a perceptually easier task under conditions of high background noise and overlapping signals.

Two lines of evidence might help clarify whether this hypothesis holds true. First, we need to know how widespread the phenomenon of interacting preferences is, and whether there are trends in the types of signal feature preferences that dominate vis-à-vis timing preferences. Second, when scoring mating success in the wild, the densities at which males displayed and females made their choices may become relevant. The hypothesis that different preference types dominate female choice as a function of chorus complexity predicts that signal-feature-based mating success will be found mainly in low-density choruses, because signal feature preferences dominate under these conditions. By contrast, in high-density choruses, signal-feature-based patterns of mating success will be different or absent, because signal timing preferences dominate. The important distinction is that variation in chorus complexity should result in different patterns of mating success, not in a pattern where, under simple conditions, mating success can be linked to female preferences, but under complex conditions, it is random.

Finally, preference interactions may be important in the maintenance of male trait variation in the population. First, the potential for communication–environment facilitated maintenance of trait variation is high. Male densities may vary temporally, either within the same night, or between nights across a breeding season. Densities may also vary spatially, with some portions of the display areas having higher densities than others. If the type of preference (timing versus feature) that dominates overall signal attractiveness shows density dependence, then the highly dynamic nature characteristic of many group displays sets the stage for very different selection regimes to act on male traits, even within a population. Second, even if preference interactions do not show density-dependent variation, the simple existence of an interaction between preference types would have the consequence of maintaining variation in male signal features as well as signal timing, because selection can favour several signal feature/timing combinations with similar overall fitness.

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