

# Sources of Selection on Signal Timing in a Tree Frog

Gerlinde Höbel\*† & H. Carl Gerhardt†

\* Abteilung Neurobiologie, Universität Ulm, Ulm, Germany

† Division of Biological Sciences, University of Missouri – Columbia, Columbia, MO, USA

## Correspondence

Gerlinde Höbel, Department of Biological Sciences, University of Wisconsin – Milwaukee, PO Box 413, Milwaukee, WI 53201, USA. E-mail: hoebel@uwm.edu

Received: March 21, 2007

Initial acceptance: May 6, 2007

Final acceptance: June 8, 2007

(S. A. Foster)

doi: 10.1111/j.1439-0310.2007.01404.x

## Abstract

Signal-timing adjustment is common in communally signaling species, and there is large variation in signal-timing formats found in nature. We conducted a survey of geographic variation in female signal-timing preferences and male signaling behavior of a tree frog to test predictions of two hypotheses about the sources of selection acting on signal-timing behavior. We found that female preferences are important in shaping male signal timing, affecting the absolute placement of signals relative to the temporal limits of female preferences. Variation in other signal characters, particularly signal period, also affects signal timing, albeit in a more complicated pattern: the influence of signal period on signal timing varied between populations. Overall, our findings indicate that the evolution of male signal-timing behavior is strongly influenced by female preferences and by an interaction with other aspects of male signaling behavior such as male signal period.

## Introduction

Advertisement signals play a central role in mate choice and pair formation in many animal species (Andersson 1994). These activities often occur in a complex social environment created when many individuals display in close proximity (Gerhardt & Huber 2002; Greenfield 2002). Within this competitive framework, males often modify their signals to increase their attractiveness, producing longer or more intense signals, or signaling at a faster rate (e.g. Gerhardt 1987; Jia et al. 2001). One of the most striking features of signaling in groups is the adjustment of the timing of signals relative to those of neighbors (Zelick & Narins 1985; Greenfield & Roizen 1993; Schwartz 1993; Grafe 1996). Signal-timing adjustment creates patterns in which males appear to coordinate their signals in group displays. However, the underlying interactions may be as competitive as in modifications of other signal traits (Greenfield 2005). Signal-timing adjustment is taxonomically widespread, and occurs in a range of signal modalities, such as visual, acoustic and chemical

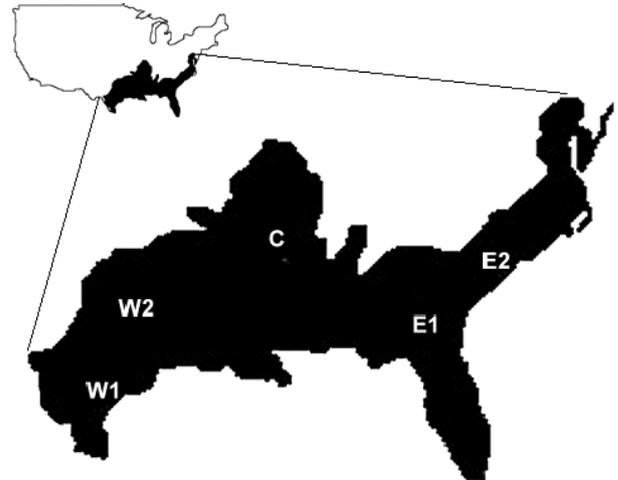
(i.e. Zelick & Narins 1985; Buck 1988; Greenfield & Roizen 1993; Lim & Greenfield 2007). The format of adjustment varies widely between species, from perfect synchrony to perfect alternation, with many intermediate forms (Gerhardt & Huber 2002; Greenfield 2005).

Two main, non-exclusive hypotheses have been proposed to explain the evolution of signal timing and its diversity (see also Schwartz 1987; Wells & Schwartz 2007). The first is female choice. When presented with two identical signals differing only in their relative timing, females of many species prefer one to the other (Gerhardt & Huber 2002; Greenfield 2005). These preferences create a 'forbidden interval' – a window around a signal in which other signals are rendered unattractive (Greenfield 1994). The hypothesis that female preferences have shaped the evolution of male signal timing makes three predictions. First, as with preferences for other signal traits, male signal timing should fit the values preferred by females (e.g. Ritchie 1996; Shaw 2000; Brooks et al. 2005; Gerhardt 2005a,b; Greenfield 2005; Rodríguez et al. 2006). In the few cases in which information

on female preferences and male signal-timing behavior is available, this prediction is met (Grafe 1999; Bosch & Márquez 2001; review in Greenfield 2005). Second, when preferences vary in strength, stronger preferences should result in lower variability in the male trait (Rodríguez et al. 2006). The third prediction is based on variation in preferences. Signal-timing preferences vary substantially between species. Females often prefer the leading of two signals (Grafe 1996; Snedden & Greenfield 1998; Bosch & Márquez 2002), but there are examples of females preferring the lagging signal (Wells & Schwartz 1984; Grafe 1999), as well as examples of no preference (Forester & Harrison 1987; Schwartz & Rand 1991). If female signal-timing preferences vary, males should vary accordingly.

The second hypothesis proposes that diversity in signal timing arises from interplay with other aspects of signaling behavior (Greenfield 1994, 2005). The basis for this hypothesis is the mechanism regulating signaling behavior. For a male, signaling by himself, a signal generator (i.e. a neural oscillator) may control signal period. The generator rises from a basal level to a trigger level, eliciting the production of a signal. The generator then returns to basal level and the process repeats, generating rhythmic signaling. Signal-timing adjustments are produced when perception of the signal of a neighboring male resets the generator to the basal level, preventing the production of a signal in an unattractive position. After an interval of inhibition, the generator rebounds and triggers a signal. Differences in the duration of the rebound may generate different signal-timing formats. For example, alternation may result when the rebound is short compared with the signal period, while synchrony may result when the rebound is relatively long (Greenfield 1994). This pattern has been observed in insects, where short signal periods (i.e. fast signal rates,  $>1$  signal  $s^{-1}$ ) are associated with synchronous signaling, whereas long signal periods (slow rates,  $<1$  signal  $s^{-1}$ ) are associated with alternation (Greenfield 1994). Thus, variation in signal period may generate diversity in signal timing. This hypothesis predicts a correlation between signal period and signal timing.

We tested these hypotheses with a survey of geographic variation in male signal timing and female preferences in green tree frogs, *Hyla cinerea*. Male *H. cinerea* adjust their signals relative to their neighbors, and female choice is affected by signal timing (Klump & Gerhardt 1992). There is geographic variation in several signal characters, notably signal period (Asquith et al. 1988; G. Höbel, unpublished



**Fig. 1:** Geographic distribution of *Hyla cinerea* in the south-eastern USA, and location of study sites (inset). The western sites are at Welder Wildlife Refuge, San Patricio Co., Texas (W1) and Richland Creek Wildlife Management Area, Freestone Co., Texas (W2). The central site is located at Private John Allen National Fish Hatchery, Lee Co., Mississippi (C). The eastern sites are located at Grand Bay Wildlife Management Area, Lowden Co., Georgia (E1) and Hobcaw Barony, Georgetown Co., South Carolina (E2).

data). We conducted playback experiments with male and female frogs at five sites throughout the range of distribution of the species (Fig. 1). We were unable to test the prediction based on preference variation, because *H. cinerea* preferences did not vary among sites (see Results). We tested the two other predictions of the female-preference hypothesis: male timing should cater to female preferences (Grafe 1999; Bosch & Márquez 2001; Greenfield 2005); and traits under stronger selection by female choice should show lower variation. Finally, we assessed the influence of signal period on signal-timing behavior by comparing the signal-timing behavior of males from populations that vary in signal period.

## Materials and Methods

### General

*Hyla cinerea* is broadly distributed in the south-eastern part of the USA (Conant & Collins 1998). We sampled five field sites across the geographic range of the species (Fig. 1). Two sites are located in the western part of the range (sites W1, W2), one is centrally located (C), and two are in the eastern part of the range (E1, E2). Although we did not make formal measurements, we estimated that chorus densities were generally high and comparable among sites. Experiments were conducted during the 2000,

2001 and 2006 breeding seasons (May to Aug.). All trials were performed in the field.

### Stimulus Design

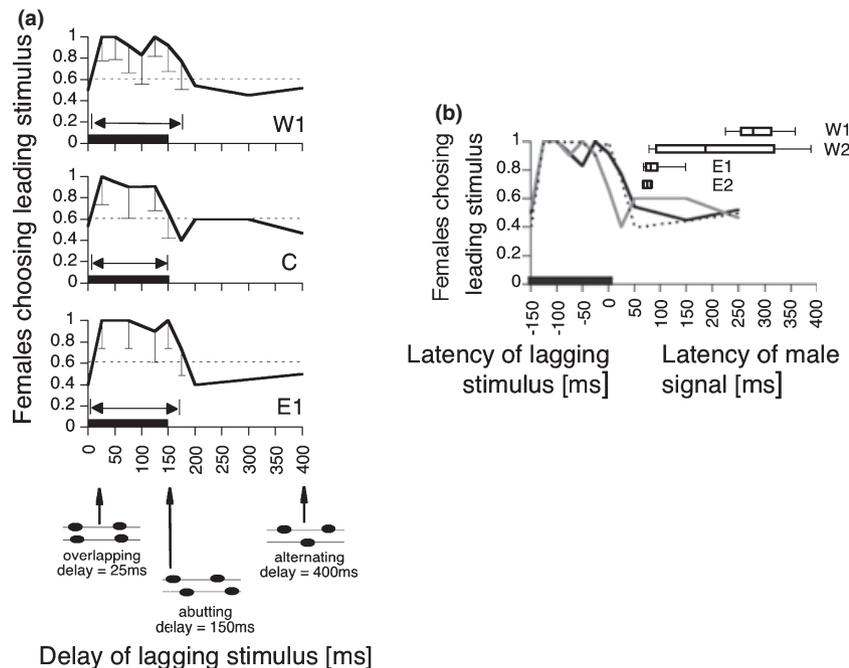
We used a custom-written DOS program (courtesy of J.J. Schwartz) to generate stimuli modeled on an *H. cinerea* advertisement signal, with characteristics set close to the mean values of the species. Stimuli had three frequency components (0.9 + 2.7 + 3.0 kHz), a rise time of 25 ms (inverse exponential), a fall time of 50 ms (inverse exponential) and a duration of 150 ms. We used editing software (CoolEdit96; Syntrillium Software Corp., Phoenix, AZ, USA) to control stimulus timing.

To generate two-choice playback trials for females, we created stereo files, with each channel containing one of two identical stimuli. We used the following values for the delay of the lagging stimulus (see Fig. 2a): 0 (complete overlap), 25, 50, 75 (50% overlap), 100, 125, 150 (abutting), 175, 200, 300, and 400 ms (perfect alternation). Thus, there were 11 two-choice stimulus combinations. For both channels the signal period was the same (800 ms). For

playbacks to males, we generated mono files that repeated the stimulus with silent intervals between 180 and 1800 ms. We recorded the sound files on a cassette tape, which we later used during field playback trials. Stimuli were presented in random order.

### Female Signal-Timing Preferences

Experiments took place either in the night the females were collected, or the following night. All females were released at the site of capture within 1 d of being tested. Trials were performed in a portable playback arena (1 × 2 m<sup>2</sup>). The sides of the arena were lined with black cloth (opaque but acoustically transparent). We placed the arena sufficiently far away from the breeding pond to ensure acoustic isolation from the chorus noise (>500 m). Two speakers (Radio Shack Optimus XTS 40, RadioShack Corporation, Fort Worth, TX, USA) were placed 180° apart, on the center of the short sides of the arena. Signals were broadcast from an IBM-compatible computer at a sampling rate of 44 kHz. The sound pressure level (SPL) of each stimulus was equalized to 85 dB SPL (re: 20 μPa) using a Lutron SL-4001 sound level meter (fast



**Fig. 2:** Signal-timing preferences of female *Hyla cinerea*. (a) The proportion (and lower 95% credible intervals) of females that approach the leading alternative. The significance criterion (lower 95% credible intervals >0.6) is indicated by the dashed horizontal line, and the resulting estimate for the duration of the forbidden interval of female preferences is indicated by the horizontal arrow. The black horizontal bar indicates the position and duration of the leading stimulus. (b) Comparison between preferences and male signal timing. The black horizontal bar denotes the duration and timing of a stimulus. Preferences (line graphs) and male signal timing (box-whisker plots) are shown in relation to this stimulus. The box-whisker plots show the top (25th percentile), median (50th percentile) and bottom quartile (75th percentile) respectively. The whiskers on the bottom extend to the 10th percentile and top 90th percentile.

root-mean-square setting; Lutron Electronic Enterprise, Teipei, Taiwan).

For testing, we placed a female in an acoustically transparent container (small round hardware cloth cage, 10-cm diameter) midway between the loudspeakers. Once the stimuli had been played back for five repetitions each, we remotely removed the lid of the release box so that the female could move freely. We observed the frogs under dim red light and scored a positive response if a female touched or moved within 10 cm of a speaker. We conducted these experiments with females from western (W1,  $n = 42$ ), central (C,  $n = 29$ ) and eastern (E1,  $n = 22$ ) sites.

We presented each of the 11 two-choice stimulus combinations to 10–23 females (mode: 10) per site, and report the proportions of females that responded to each of the alternatives, along with the 95% lower credible limits on these proportions (Gerhardt 1992). We consider a preference to be statistically significant if the lower 95% credible limit on the proportion of females choosing one alternative is  $>0.60$ ; results meeting this criterion would also be significant at the 0.05 level in a two-tailed binomial test.

To evaluate geographic variation in female preferences, we conducted a two-step statistical analysis. First, we tested for an effect of site and for linear and quadratic effects of stimulus duration and their interaction with site (Table 1; full model). In this test, the magnitude of F-ratio for stimulus delay suggests that low power, rather than no effect was responsible for the non-significant result. To increase power, we removed the non-significant quadratic and interaction terms (whose low F-ratios did indicate no effect) and tested for the effect of site, stimulus duration and their interaction (Table 1; simplified model). The interaction term tests for differences in preferences between sites (see Olviedo & Wagner 2004; Rodríguez et al. 2006).

**Table 1:** Female signal-timing preferences in three sites (W1, C and E1)

Factor	(a) Full model	(b) Simplified model
Study site	$F_{2,19} = 0.56, p = 0.58$	$F_{2,22} = 0.43, p = 0.65$
Stimulus delay	$F_{1,19} = 2.72, p = 0.12$	$F_{1,22} = 6.92, p = 0.015$
Interaction	$F_{2,19} = 0.08, p = 0.92$	$F_{2,22} = 0.05, p = 0.95$
Quadratic	$F_{1,19} = 0.45, p = 0.51$	
Quadratic $\times$ site	$F_{2,19} = 0.19, p = 0.82$	

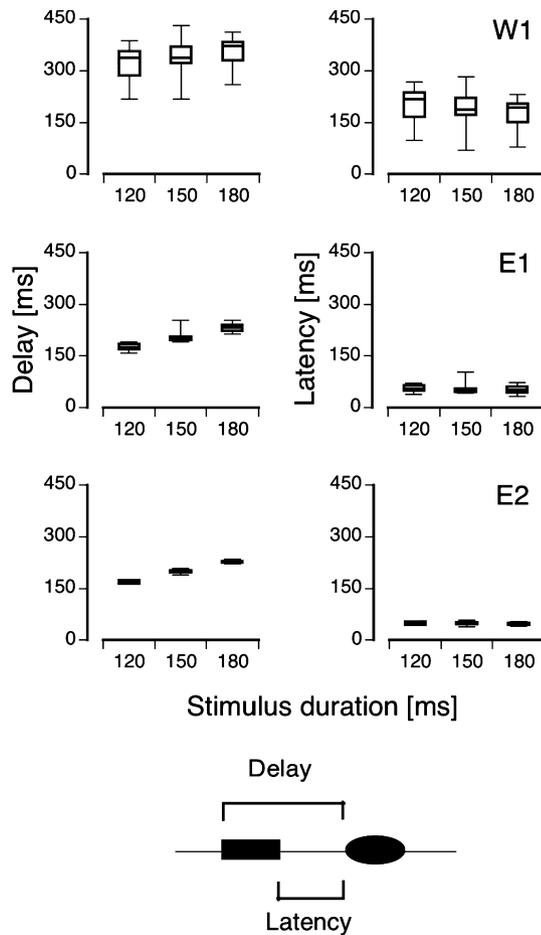
We conducted a two-step analysis in which we first tested for effect of site and linear and quadratic effects of stimulus duration and their interaction with site (full model). After verifying that the quadratic terms were not significant, we removed the quadratic term and the interaction to improve power and repeated the model testing only for the effect of site, stimulus duration and their interaction (simplified model).

### Male Signal-Timing Behavior

We elicited male responses with playbacks of synthetic stimuli. We selected an isolated male (nearest neighbor  $>10$  m) that was signaling regularly. We then presented stimuli with a speaker and microphone, mounted on tripods, placed 2 m away from the male. Each male was presented with 100 stimulus repetitions. The playback set-up consisted of a RadioShack Optimus XTS-40 speaker (RadioShack Corporation, Fort Worth, TX, USA), driven by a Sony WM-D6C recorder (Sony Corporation, Tokyo, Japan) and amplified by a RadioShack Optimus XL-50 Stereo Amplifier (RadioShack Corporation, Fort Worth, TX, USA). The signal from the playback recorder was split so it could be recorded on one channel of a Sony TC-D5M stereo tape recorder (Sony Corporation, Tokyo, Japan), which on the other channel was recording the focal male with an Audio-Technica US ATR55 microphone (Audio-Technica U.S., Inc., Stow, OH, USA).

Because males may use the beginning or the end of a stimulus to time their own signal, we first determined which stimulus features serve as the trigger for signal timing. We compared signal timing in response to stimuli with 120, 150 and 180 ms duration. These values span the range of variation of signal duration in *H. cinerea* (G. Höbel, personal observation). Other stimulus features (rise/fall time and spectral composition) were set to average values (see above), and stimuli were presented in random order and with 800 ms of silence between consecutive stimuli. We measured the time from the beginning of the stimulus to the beginning of the following male signal (delay, Fig. 3, left column), as well as from the end of the stimulus to the beginning of the following male signal (latency, Fig. 3, right column). If males use the beginning of the stimulus to time their signals, then delay should not be affected by stimulus duration; if males use the end of the stimulus, latency should not be affected. We conducted this experiment in one western (W1) and two eastern sites (E1, E2). We tested 12 males at W1, 12 males at E1 and 18 males at E2. We measured seven to 49 (mode 35) responses per male for each stimulus. Once we determined the stimulus feature that influenced male signal timing, we compared geographic differences in signal timing using this value.

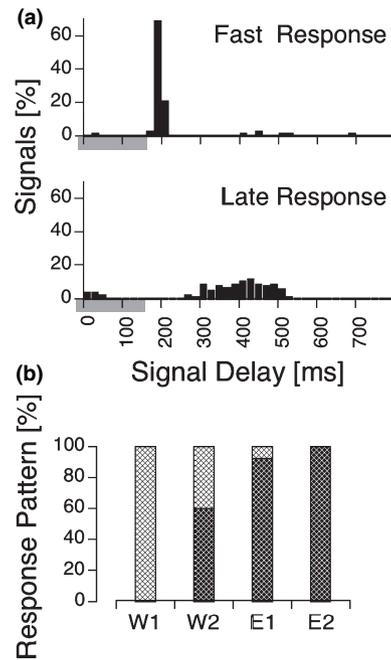
To describe geographic variation in male signal timing we conducted playback experiments at two western (W1 and W2) and two eastern (E1 and E2) sites. We tested 10 males at W1, 10 males at W2, 13 males at E1 and 17 males at E2. We measured



**Fig. 3:** Changes in delay (first column) and latency (second column) in response to stimuli of different duration (see Table 2). Shown are the top (25th percentile), median (50th percentile) and bottom quartile (75th percentile) respectively. The whiskers on the bottom extend to the 10th percentile and top 90th percentile.

responses to 13–100 (mode 60) stimuli per male. The average SPL of the stimuli at the point of the male was  $83 \pm 2$  dB, and the average temperature at the signaling site of the male was  $24 \pm 2$  °C. There was no significant difference in stimulus amplitude ( $F_{3,49} = 0.48$ ,  $p = 0.70$ ) nor temperature ( $F_{3,49} = 0.43$ ,  $p = 0.43$ ) between sites.

We digitized (at 22.5 kHz) and analyzed the recordings of male signal-timing responses with CoolEdit96 (Syntrillium Software Corp., Phoenix, AZ, USA). We measured the interval between the beginning of a stimulus to the beginning of the following signal of the male, and plotted signal delay histograms for visual inspection of the pattern of male responses (Fig. 4a). We obtained the following data for each male: (1) the proportion of signals that were overlapped by playback stimuli and occupied



**Fig. 4:** Within- and between-site variations in signal timing in *Hyla cinerea*. (a) Examples of signal delay histograms of the two response patterns. Shown is the proportion of signals that began within each of 40 consecutive 20-ms bins, bin one starting at the onset of the playback stimulus (beginning and duration of stimulus is indicated by gray horizontal bar). Some males strongly entrained to the playback stimulus, and placed most calls within a short time window quickly after the end of the stimulus (top). Other males placed signals within a broad window at later delays (bottom). (b) Distribution of response patterns across study sites (clear pattern – late response, dark pattern – fast response).

a lagging position; (2) the timing of the earliest signal the male gave after the end of a stimulus (post-stimulus suppression); and (3) the timing at which the male placed most of its signals (modal latency; see Fig. 4). We consider suppression and latency to be different traits, because the timing of the first signal after a stimulus might be due to a physiological, stimulus-induced suppression response, whereas the timing of most signals could be due to males' choosing a particular timing relationship between the stimulus and their signal. We calculated post-stimulus suppression and modal latency by noting the minimum delay of signals that did not overlap with the stimulus as well as the modal signal delay of each male. After verifying that males time their signal from the end of stimuli (Fig. 3, Table 2), we converted the values obtained for minimum and modal signal delay to latency estimates by subtracting 150 ms (the duration of the stimuli used in the trials).

**Table 2:** Effect of variation of stimulus duration on the delay and latency of the signals elicited from *Hyla cinerea* males in three test populations (W1, E1 and E2)

Factor	Delay	Latency
Study site	$F_{2,117} = 176.43, p < 0.001$	$F_{2,117} = 176.43, p < 0.001$
Stimulus duration	$F_{2,117} = 18.96, p < 0.001$	$F_{2,117} = 0.96, p = 0.39$
Interaction	$F_{4,117} = 0.54, p = 0.70$	$F_{4,117} = 0.54, p = 0.70$

We calculated a one-way ANOVA to compare responses between sites. Proportional data were arcsine transformed before statistical analysis.

### Preference – Signal-Timing Correspondence

To test the prediction that male signal timing should match female preferences, we characterized the forbidden interval in the preferences and compared it with the timing of male responses to playback stimuli. We evaluated: (1) whether males overlap fewer signals with the playback stimuli than expected if they were unaffected by the playback. We obtained the expected value from the average duty cycle (proportion of the cycle that is occupied by a signal) of a sample of 30 males recorded at each site. (2) The duration of the post-stimulus suppression. (3) The duration of modal latency. We used a Welch-ANOVA (which takes heterogeneity of variance into account) to test for among-site variation.

To test the prediction that the trait under stronger selection from female preferences should be less variable, we compared the variance in post-stimulus suppression and modal latency. Post-stimulus suppression results in signal placement closer to the forbidden interval than modal latency. Thus, post-stimulus suppression should have lower variance than modal latency. We used one-way ANOVA's to obtain the between-site and within-site variance components for post-stimulus suppression and modal latency (Sokal & Rohlf 1997, pp. 211–212). We tested whether the variance component for post-stimulus suppression is lower than for modal latency with F-ratios. Because the measurements for each trait came from the same individuals, the degrees of freedom for the numerator and denominator of the F-ratios are the same. To scale the variance components to the same units, we divided each variance component by the mean of the trait. With this scaling, the expectation under the null hypothesis that the variance component for post-stimulus suppression is not lower than for modal latency is that  $F = 1$ .

### Relationship Between Male Signal Period and Signal Timing

We recorded the undisturbed signaling behavior of each male for 1 min before starting the playback trial. We describe signal period with the time from the beginning of one signal to the beginning of the following signal of a male, and calculate mean values based on the measurement of 10 signals. To test whether signal timing (represented by modal latency) was affected by signal period, we first calculated a two-way ANOVA that included data from all males from all four test sites. This test indicated that the influence of signal period on signal latency varied between study sites (significant interaction; Table 4). We thus tested the correlation between the two traits in each population separately with Pearson product moment correlations.

## Results

### Female Signal-Timing Preferences

Females from all sites showed preferences on the basis of signal timing. When confronted with identical but overlapped stimuli (delays from 25 to 125 ms), females preferred the stimulus in leading position. Females ceased to do so (lower 95% credible values <0.6) once the lagging stimuli were presented at relative delays of 150 ms (site C) or 175 ms (sites W1 and E1). These delays correspond to stimuli given in abutting position or stimuli with a short silent period between them (Fig. 2a). Females did not discriminate between stimuli presented simultaneously (0 ms delay), i.e. overlapped but without relative timing information.

This female preference creates a 'forbidden interval' during which males should avoid producing a signal. The duration of the forbidden interval was as long or a little longer as the duration of the leading stimulus and did not vary significantly between sites (Fig. 2b, see non-significant site  $\times$  stimulus delay interaction in Table 1).

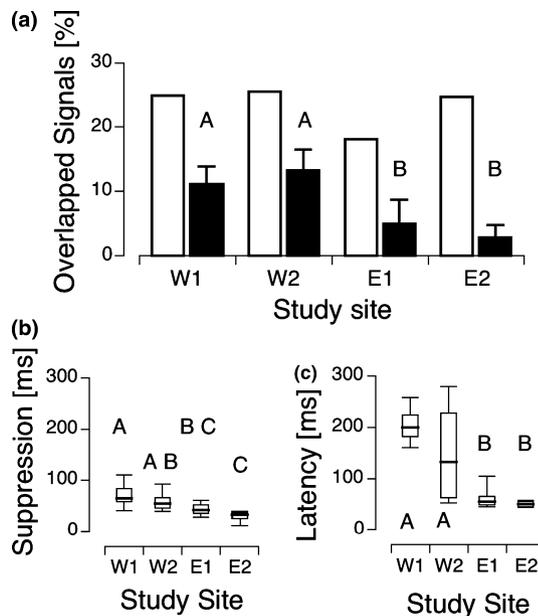
### Male Signal Timing

In all three populations tested in this preliminary assay, stimulus duration influenced stimulus delay, but not stimulus latency (Fig. 3; Table 2). From this result, we conclude that males use the end of the stimulus to time their signals. Accordingly, we used post-stimulus suppression and modal latency to describe signal-timing behavior of male *H. cinerea*.

**Table 3:** Test of geographic variation in expected/observed amount of signal overlap

Factor		
Study site	$F_{3,159} = 47.42$	$p < 0.0001$
Expected/observed overlap	$F_{1,159} = 619.83$	$p < 0.0001$
Interaction	$F_{3,159} = 27.14$	$p < 0.0001$

Males showed substantial within- and between-site variation in signal overlap and signal-timing adjustments. First, although for all tested males the observed number of calls that overlapped with the stimulus was much lower than expected (Table 3), signal overlap was overall significantly rarer in eastern sites than in western sites (Fig. 5a, Table 3). Second, some males placed most of their signals within a brief window shortly after the end of the stimulus, a pattern of time-locked response referred to as entrainment (Fig. 4a, top). Other males placed their signals much later, in a wider window (Fig. 4a, bottom). Although there were males that placed calls in the fast-response pattern as well as the late-response pattern, there was not a smooth continuum between



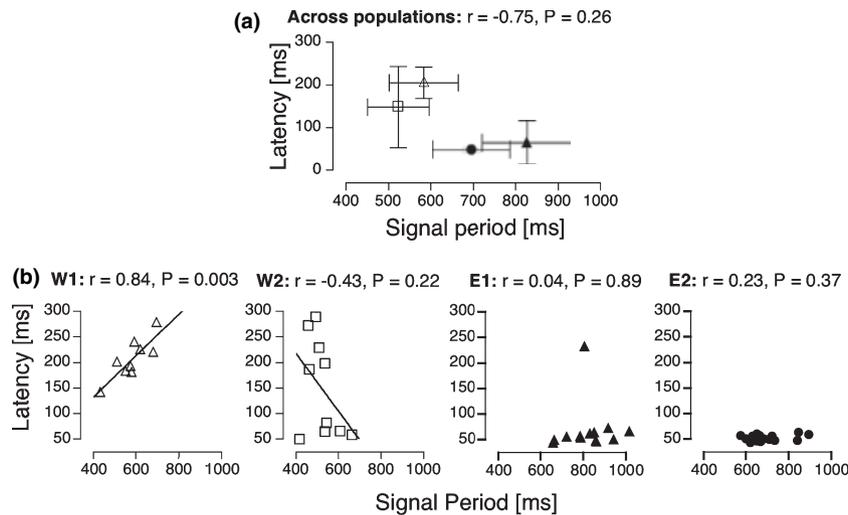
**Fig. 5:** Geographic variation in *Hyla cinerea* signal-timing traits. (a) Males in all populations overlap less signals with the playback stimulus than expected. Open bars indicate the expected level of signal overlap, the filled bars show the mean proportion (+SD) of signal overlap observed in each site (see Table 3). Letters indicate results of Tukey's post hoc comparisons. (b) Post-stimulus suppression ( $F_{3,49} = 12.17$ ,  $p < 0.001$ ) and (c) modal latency ( $F_{3,49} = 23.02$ ,  $p < 0.001$ ) also varied. Shown are the top (25th percentile), median (50th percentile) and bottom quartile (75th percentile) respectively. The whiskers on the bottom extend to the 10th percentile and top 90th percentile.

the two patterns. Rather, males either adhered mainly to one format or the other, which ultimately led us to group males into only two response format categories. The proportion of males responding in each of these formats varied between sites ( $\chi^2_3 = 34.2$ ,  $p < 0.001$ ; Fig. 4b). As a consequence of this variation in the proportion of males that signaled in the two formats, there were differences in the average values of post-stimulus suppression and modal latency between populations. Post-stimulus suppression and modal latency were longer in western sites than in eastern sites. Whereas variation in post-stimulus suppression was clinal and of relatively small magnitude (Fig. 5b), variation in modal latency was more pronounced and showed a bimodal distribution (Figs 5c and 6a): males either signal with a latency of approx. 50 ms, or of 200–300 ms.

#### Preference – Signal-Timing Correspondence

We compared the forbidden interval of females with the distribution of signal-timing traits. We found a remarkable correspondence between male behavior and female preferences (Fig. 2b). In all populations, males produced fewer overlapped signals than expected if they did not attend to the stimulus (Figs 4a and 5a). Signal overlap generally occurred only during the initial portion of the stimulus (Fig. 4a). There was a period of complete signal suppression, which not only extended for the remaining portion of the stimulus, but also included a post-stimulus suppression period of 30–70 ms (Figs 4a and 5b). Finally, males placed most of their signals at least 50 ms after the end of the playback stimulus (Figs 4a and 5c).

Post-stimulus suppression (Welch-ANOVA:  $F_{3,20} = 11.1$ ,  $p = 0.002$ ) and modal latency ( $F_{3,16.7} = 56.4$ ,  $p < 0.001$ ) showed significant between-site variation (Fig. 5b, c). We thus performed two separate tests of the prediction that the variance in post-stimulus suppression should be lower than the variance in modal latency: one test for between-site variation and one for within-site variation. The between-site variance component was lower for post-stimulus suppression than for modal latency, and the comparison was marginally significant ( $F_{3,3} = 6.94$ ;  $0.1 > p > 0.05$ ). The magnitude of the F-ratio indicates that the lack of significance was due to low power (as there were only four populations) and not caused by a small difference in the variance components. The within-site variance component for post-stimulus suppression was significantly lower than for modal latency ( $F_{46,46} = 3.52$ ,  $p < 0.001$ ).



**Fig. 6:** Relationship between signal period and modal latency. (a) Average ( $\pm$ SD) signal period and latency across sites ( $\Delta$  W1,  $\square$  W2,  $\blacktriangle$  E1,  $\bullet$  E2). (b) The relationship between period and modal latency varied between sites.

### Relationship Between Male Signal Period and Signal Timing

There was substantial geographic variation in signal period and modal latency (Fig. 6a, b). Across populations, mean period was negatively correlated with mean modal latency (Fig. 6a). This correlation was not significant, but with  $n = 4$  sites, statistical power is adequate ( $1 - \beta \geq 0.80$ ) only for  $r \geq 0.98$  (Cohen 1988; Zar 1999, p. 385). Further, to explore the relationship between signal period and latency, we tested the effect of period and site on modal latency. There was a significant effect of site and an interaction between site and period (Table 4), suggesting geographic variation in the relationship between period and modal latency. We thus examined the relationship within each site. In the western sites, the two traits were correlated, with one site showing a positive correlation and the other site showing a negative correlation (Fig. 6b, left plots; only one correlation was significant, but with  $n = 10$  individuals per site, statistical power is adequate only for  $r \geq 0.79$ ; Cohen 1988; Zar 1999, p. 385). We thus focused on the effect size of the correlations: in W1 it was strong and positive; in W2 it was moderate and negative. In the eastern sites there was no correlation.

**Table 4:** Effect of signal period and test site on modal latency

Factor		
Study site	$F_{3,41} = 15.56$	$p < 0.001$
Signal period	$F_{1,41} = 0.11$	$p = 0.74$
Interaction	$F_{3,41} = 3.23$	$p = 0.032$

### Discussion

We conducted a survey of geographic variation in female signal-timing preferences and male signaling behavior to evaluate hypotheses about the sources of selection and variation in signal timing. We tested two predictions of the hypothesis that female preferences have been important in shaping male signal timing: (1) male signal placement should match the temporal limits of female preferences; and (2) the male trait under stronger selection from female preferences should have lower between- and within-site variances. We found strong support for both predictions, indicating that in *H. cinerea* female choice is an important source of selection on male signal timing. However, there was no between-site variation in signal-timing preferences, so female choice does not appear to account for the observed variation in male signal timing. A previous study of *H. cinerea* (Klump & Gerhardt 1992) found that the forbidden interval extended for 120 ms beyond the end of the overlapped signal, a substantially longer interval than detected for any of the populations tested in the present paper. This difference may suggest geographic variation in signal-timing preferences. However, an alternative explanation is that the results may have been affected by stimulus duration (180 ms long in Klump & Gerhardt 1992; 150 ms long in the present paper). We have preliminary data suggesting that stimulus duration may influence the length of the forbidden interval (G. Höbel, unpubl. data).

We also tested the hypothesis that signal period influences signal timing. The magnitude and sign of the correlation between signal period and timing

varied across sites, suggesting that there is interplay between signal timing and period, which is influenced by additional sources of diversity. One potential factor may be variation in female preferences for different signal traits. *Hyla cinerea* females show preferences on the basis of signal frequency, duration, period and amplitude (Gerhardt 1987; Höbel & Gerhardt 2003). If there is geographic variation in these preferences, it may result in a trade-off between the effectiveness of different signal traits. For example, females in some sites may attend more to signal period than to timing of calls relative to those of a neighbor, whereas females in other sites may attend more to timing than to period. The result could be differential selection on male signaling, selecting either for short signal periods or accurate signal timing. Consistent with this possibility are differences in male signaling behavior that we observed in our study sites. Males from western populations had the shortest signal periods and produced the highest proportion of overlapped signals, while males from eastern sites had longer signal periods and more often avoided signal overlap. If in some populations there is a premium on fast signal rates, while in others there is a premium on overlap avoidance; this variation in selection on signaling behavior may promote divergence.

In conclusion, our findings indicate that the evolution of signal-timing behavior is strongly influenced by both female preferences and an interaction with other aspects of male signaling behavior. The multiplicity of influences on signal timing may be an important cause of divergence in this spectacular aspect of group-signaling behavior.

### Acknowledgements

This work was supported by travel and dissertation grants from the German Academic Exchange Service and Graduiertenförderung des Landes Baden-Württemberg, Germany, to GH. HCG's research was funded by the National Science Foundation (IBN IBN-9873669) and Public Health Service (DHHS R01 DC05760). We thank G. Ehret for loan of equipment and J. J. Schwartz for use of his signal generation program. M. D. Greenfield, R. L. Rodriguez S., and two anonymous reviewers provided invaluable help improving earlier drafts of the manuscript.

### Literature Cited

Andersson, M. 1994: Sexual Selection. Princeton Univ. Press, Princeton, NJ.

- Asquith, A., Altig, R. & Zimba, P. 1988: Geographic variation in the mating call of the green treefrog *Hyla cinerea*. *Am. Midl. Nat.* **119**, 101–110.
- Bosch, J. & Márquez, R. 2001: Call timing in male–male acoustical interactions and female choice in the midwife toad *Alytes obstetricans*. *Copeia* **2001**, 169–177.
- Bosch, J. & Márquez, R. 2002: Female preference function related to precedence effect in an amphibian anuran (*Alytes cisternasii*): tests with non-overlapping calls. *Behav. Ecol.* **13**, 149–153.
- Brooks, R., Hunt, J., Blows, M. W., Smith, M. J., Bussière, L. F. & Jennions, M. D. 2005: Experimental evidence for multivariate stabilizing selection. *Evolution* **59**, 871–880.
- Buck, J. 1988: Synchronous rhythmic flashing in fireflies. *II. Q. Rev. Biol.* **63**, 265–298.
- Cohen, J. 1988: *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. Lawrence Erlbaum Associates, Hillsdale, NJ.
- Conant, R. & Collins, J. T. 1998: *A Field Guide to Reptiles and Amphibians: Eastern and Central North America*. Houghton Mifflin Co., Boston, MA.
- Forester, D. C. & Harrison, W. K. 1987: The significance of antiphonal vocalization by the spring peeper, *Pseudacris crucifer* (Amphibia, Anura). *Behavior* **103**, 1–15.
- Gerhardt, H. C. 1987: Evolutionary and neurobiological implications of selective phonotaxis in the green treefrog, *Hyla cinerea*. *Anim. Behav.* **35**, 1479–1489.
- Gerhardt, H. C. 1992: Conducting playback experiments and interpreting their results. In: *Playback and Studies of Animal Communication* (McGregor, P. K., ed.). Plenum Press, New York, pp. 59–78.
- Gerhardt, H. C. 2005a: Acoustic spectral preferences in two cryptic species of grey treefrogs: implications for mate choice and sensory mechanisms. *Anim. Behav.* **70**, 39–48.
- Gerhardt, H. C. 2005b: Advertisement-call preferences in diploid-tetraploid treefrogs (*Hyla chrysoscelis* and *Hyla versicolor*): implications for mate choice and the evolution of communication systems. *Evolution* **59**, 395–408.
- Gerhardt, H. C. & Huber, F. 2002: *Acoustic Communication in Insects and Anurans; Common Problems and Diverse Solutions*. Univ. of Chicago Press, Chicago, IL.
- Grafe, T. U. 1996: The function of call alternation in the African reed frog (*Hyperolius marmoratus*): precise call timing prevents auditory masking. *Behav. Ecol. Sociobiol.* **38**, 148–158.
- Grafe, T. U. 1999: A function if synchronous calling and a novel female preference shift in an anuran. *Proc. R. Soc. Lond. B* **266**, 2331–2336.
- Greenfield, M. D. 1994: Cooperation and conflict in the evolution of signal interactions. *Annu. Rev. Ecol. Syst.* **25**, 97–126.

- Greenfield, M. D. 2002: *Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication*. Oxford Univ. Press, Oxford, UK.
- Greenfield, M. D. 2005: Mechanisms and evolution of communal sexual displays in arthropods and anurans. *Adv. Study Behav.* (Slater, P., Snowdon, C., Roper, T., Brockmann, H. J. & Naguib, M., eds) **35**, 1–62.
- Greenfield, M. D. & Roizen, I. 1993: Katydid synchronous chorusing is an evolutionary stable outcome of female choice. *Nature* **364**, 618–620.
- Höbel, G. & Gerhardt, H. C. 2003: Reproductive character displacement in the communication system of green tree frogs (*Hyla cinerea*). *Evolution* **57**, 894–904.
- Jia, F.-Y., Greenfield, M. D. & Collins, R. D. 2001: Ultra-sonic signal competition among male wax moths. *J. Insect Behav.* **14**, 19–33.
- Klump, G. M. & Gerhardt, H. C. 1992: Mechanisms and function of call-timing in male–male interactions in frogs. In: *Playback and Studies of Animal Communication* (McGregor, P. K., ed). Plenum Press, New York, pp. 153–174.
- Lim, H. & Greenfield, M. D. 2007: Female pheromonal chorusing in an arctiid moth, *Utetheisa ornatrix*. *Behav. Ecol.* **18**, 165–173.
- Olviedo, A. E. & Wagner, W. E. Jr 2004: Signal components, acoustic preference functions and sexual selection in a cricket. *Biol. J. Linn. Soc.* **83**, 461–472.
- Ritchie, M. G. 1996: The shape of female mating preferences. *Proc. Natl Acad. Sci. U.S.A.* **93**, 14628–14631.
- Rodríguez, R. L., Ramaswamy, K. & Coccoft, R. B. 2006: Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. *Proc. R. Soc. Lond.* **273**, 2585–2593.
- Schwartz, J. J. 1987: The function of call alternation in anuran amphibians: a test of three hypothesis. *Evolution* **41**, 461–471.
- Schwartz, J. J. 1993: Male calling behavior, female discrimination and acoustic interference in the neotropical treefrog *Hyla microcephala* under realistic acoustic conditions. *Behav. Ecol. Soc.* **32**, 401–414.
- Schwartz, J. J. & Rand, A. S. 1991: The consequences for communication of call overlap in the tungara frog, a neotropical anuran with a frequency-modulated call. *Ethology* **89**, 73–83.
- Shaw, K. L. 2000: Interspecific genetics of mate recognition: inheritance of female acoustic preferences in Hawaiian crickets. *Evolution* **54**, 1303–1312.
- Snedden, W. A. & Greenfield, M. D. 1998: Females prefer leading males: relative call timing and sexual selection in katydid choruses. *Anim. Behav.* **56**, 1091–1098.
- Sokal, R. R. & Rohlf, F. J. 1997: *Biometry*. W. H. Freeman and Company, New York.
- Wells, K. D. & Schwartz, J. J. 1984: Vocal communication in a neotropical treefrog, *Hyla ebraccata*: advertisement calls. *Anim. Behav.* **32**, 405–420.
- Wells, K. D. & Schwartz, J. J. 2007: The behavioral ecology of anuran communication. In: *Hearing and Sound Communication in Amphibians* (Narins, P. M., Feng, A. S., Fay, R. R. & Popperr, A. N., eds). Springer Verlag, New York, pp. 44–86.
- Zar, J. H. 1999: *Biostatistical Analysis*, 4th edn. Prentice-Hall, Upper Saddle River, NJ.
- Zelick, R. & Narins, P. M. 1985: Characterization of the advertisement call oscillator in the frog *Eleutherodactylus coqui*. *J. Comp. Physiol. A* **156**, 223–229.