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Variation in signal timing behavior: implications for male attractiveness and sexual selection

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Abstract Mate choice often takes place in group settings, such as leks or choruses, in which numerous individuals display and compete for mates simultaneously. In addition to well-known preferences for male traits like size and signaling rate, females of group-displaying species often show preferences that are based on the relative timing of male signals, generally preferring the leading signal. Variation in male signal timing behavior may therefore affect male attractiveness and, ultimately, reproductive success. I used acoustic communication in green treefrogs (*Hyla cinerea*) to assess the amount of signal timing variation found in natural choruses and to test hypotheses about the sources of variation in signal timing behavior. I recorded dyads of vocally interacting males in the field to describe patterns of variation in signal timing behavior. Incorporating information about female signal timing preferences, I also assessed the amount of variation in signal timing that is likely to be selectively neutral, as well as the amount that is likely under selection by female choice because it involves attractive or unattractive signal placement. I show that there is considerable variation in signal timing behavior, particularly involving neutral signal timing placement. I also show that between-male variation in other traits (size, signal period) is partly linked to variation in signal timing behavior and discuss these findings in terms of male attractiveness and sexual selection in group-displaying organisms.

Introduction

Mate choice often takes place in group settings, such as leks or choruses, in which numerous individuals display and compete for mates simultaneously (Höglund and Alatalo 1995; Gerhardt and Huber 2002; Greenfield 2002). These aggregations create complex social environments that pose special challenges to effective communication. For example, signal interference from background noise or individual signals may obscure spectral or temporal signal features important for mate choice and impair signal detection (Ehret and Gerhardt 1980; Gerhardt and Klump 1988; Wollerman 1999; Bee 2007) or the ability of females to discriminate among different conspecific signals (Schwartz and Gerhardt 1989; Schwartz et al. 2001; Wollerman and Wiley 2002; Bee 2008).

Signal overlap by itself can influence mate choice, and females of group-displaying species often show preferences that are based on the timing at which a male's signal is perceived relative to his neighbor's signal (reviewed in Gerhardt and Huber 2002; Greenfield 2002, 2005). Signal timing preferences have been found in a wide range of taxa and signal modalities, including acoustically signaling anuran amphibians, acoustic insects like katydids and grasshoppers, and visually signaling fireflies and fiddler crabs (Minckley and Greenfield 1995; Grafe 1996; Snedden and Greenfield 1998; Vencl and Carlson 1998; Höbel and Gerhardt 2007, Reaney et al. 2008). In general, females prefer leading signals (Dyson and Passmore 1988; Greenfield et al. 1997; Klump and Gerhardt 1992; Vencl and Carlson 1998; Whitney and Krebs 1975; but see Forester and Harrison 1987; Grafe 1999).

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Once the occurrence of a leader preference is identified (generally tested in trials in which signals overlap by 50%), a seemingly straightforward way of describing male signal timing behavior and assessing its importance for sexual selection is to score the proportion of a male's signals in leading, alternating, and lagging position. This, however, assumes that leading signals are equivalent to attractive ones. To verify that this is the case, it is useful to obtain preference functions. Here, preferences are tested across the full range of possible signal timing positions, from complete overlap over several degrees of lead–lag relationships to perfect alternation. Such preference functions have revealed that, in some species, leader preference only extends for the duration of actual signal overlap (Fig. 1a; e.g., Grafe 1996; Höbel and Gerhardt 2007), while in other species the leader preference extends to include non-overlapping leading signals (Fig. 1b; e.g., Greenfield and Roizen 1993; Minckley and Greenfield 1995; Snedden and Greenfield 1998; Bosch and Márquez 2002). However, in either case, the preference for the leading signal creates a "forbidden interval" during which a follower signal will be unattractive (Greenfield 1994). Consequently, an important conclusion derived from signal timing preference functions is that during signaling interactions there are (1) attractive signal timing positions (i.e., leading signals within the forbidden interval; Fig. 1a, top trace), (2) unattractive positions (i.e., lagging signals within the forbidden interval; Fig. 1a, bottom trace), and (3) selectively neutral positions (i.e., all positions outside the forbidden interval, irrespective of whether signals are leading, alternating, or lagging; Fig. 1b, c).

In species in which females have signal timing preferences, the average rate of signal overlap tends to be low (Grafe 1996; Greenfield and Snedden 2003; Höbel and Gerhardt 2007), suggesting that males are good at avoiding signal overlap. Yet there is often substantial within- and among-population variation in signal timing behavior, including overlap (Grafe 1996; Höbel and Gerhardt 2007). Variation in signal timing behavior has the potential to affect male attractiveness, but this depends on where the variation is concentrated. If most variation involves neutral signal timing positions, between-male differences in signal timing behavior should have little, if any, effect on male attractiveness. If, however, most variation involves attractive vs. unattractive signal positions, then between-male differences in signal timing behavior could strongly affect male attractiveness. Consequently, the first step in evaluating the effect of variation in signal timing behavior on male attractiveness is to determine which proportion of a male's signals are in attractive, neutral, or unattractive signal timing positions.

The next step in evaluating the effect of signal timing behavior on male attractiveness is to identify the sources of
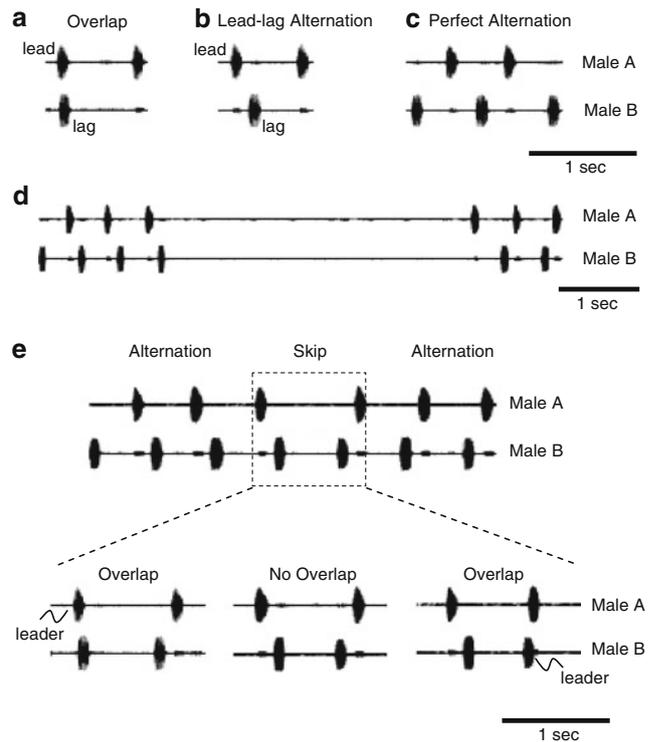

Fig. 1 Waveform displays showing relative timing positions of *H. cinerea* signals. **a–c** Examples of rhythmic signal exchanges, in which signals are traded in one-on-one fashion, resulting in **a** signal overlap, in which the lagging signal of one male (here B) is overlapped by the signal of the other male (here A); **b** lead–lag alternation, in which the signal of one male (here B) closely follows the signal of the other male (here A) but does not overlap it; and **c** perfect alternation, in which the signal of one male is placed exactly between two consecutive signals of the other male. **d–e** Examples of arhythmic signal exchanges. **d** Example where males take breaks and partition their signaling activity into discrete bouts. **e** Example of a skip, where one male (here A) leaves out a signal and the other (here B) produces two signals before male A signals again. The three examples below show the relative timing of signals possible during skips

variation in signal timing behavior. One potential source of variation arises from changes in the rhythmicity of signal exchanges. In many anuran species, signal timing interactions are very rhythmic, with two nearest neighbors trading signals in a regular A–B–A–B pattern (Fig. 1a–c; Schneider et al. 1988; Bosch and Márquez 2001; but see Christensen-Dalsgaard et al. 2002). This regular rhythmicity is sometimes interrupted, however, and two common patterns of interruptions are "breaks" and "skips" (Greenfield 1983; Schneider et al. 1988; Bosch and Márquez 2001). Breaks are longer periods of silence during which one or both males refrain from signaling between bouts of signaling activity (see Fig. 1d). Skips are instances during which one male (A) skips a signal, while the other (B) produces two signals before the first male signals again (i.e., A–B—B–A) (Fig. 1e). Regular rhythmicity may help males adjust to each other's signaling rhythms, thus reducing

unattractive signal overlap, while changes in rhythmicity may increase variability in signal timing.

Another source of variation in signal timing behavior may arise from variation in other male traits like body size or signal period. Female choice in group-signaling species is often affected by signal timing preferences (e.g., Greenfield 2005), but other types of preferences also play a role. For example, preferences for signal features like lower frequency, which is often correlated to larger body size (review Gerhardt and Huber 2002), and preferences for shorter signaling periods are widespread (review Gerhardt and Huber 2002; Ryan and Keddy-Hector 1992). Whether males with otherwise attractive traits also produce more signals in attractive signal timing positions is not well understood (but see Richardson et al. 2008) but of potential importance for our understanding of signal evolution in group-signaling species. If larger/faster signaling males also produce more attractively timed signals, selection on signal timing and other male traits would be parallel. By contrast, if there is no correlation, or a negative correlation, selection on different types of traits could be unrelated or even disruptive.

Here I use acoustic communication in green treefrogs, *Hyla cinerea*, to assess the amount of signal timing variation found in nature and to test hypotheses about the sources of variation in signal timing behavior. As in many anurans, *H. cinerea* rely largely on long-range acoustic signals for pair formation (Gerhardt 1987), and relative signal timing is an important component of their signaling behavior (Klump and Gerhardt 1992; Höbel and Gerhardt 2007). I recorded dyads of freely signaling *H. cinerea* males in the field and describe variation in signal timing in terms of leading, alternating, and lagging signal placement. In addition, the signal timing preference functions available for *H. cinerea* (Höbel and Gerhardt 2007) allowed me to assess the amount of variation in male signal timing behavior that is likely to be neutral vs. variation in signal timing that is attractive or unattractive and thus under selection by female choice. I found that there is considerable variation in signal timing behavior and that most of it involves neutral signal timing positions. I also found that there is variation in the proportion of attractive and unattractive signals and that changes in signaling rhythmicity as well as the between-male variation in other male traits (size, signal period) are linked to variation in signal timing behavior.

Methods

Study sites and species

H. cinerea is a common inhabitant of lakes, ponds, and swamps in the southeastern United States (Conant and Collins 1998). During spring and summer, males aggregate to advertise for females, creating large, dense choruses. Males start signaling shortly after dark and signal for 2–4 h each night. The advertisement signal of *H. cinerea* consists of a single pulse and is repeated once or twice per second. Females prefer leading signals, and the forbidden interval extends mainly for the duration of actual signal overlap (Höbel and Gerhardt 2007). Male *H. cinerea* avoid signal overlap with randomly presented synthetic stimuli and place signals shortly after the end of the stimulus and thus outside the forbidden interval (Höbel and Gerhardt 2007).

I studied male signal timing behavior at Welder Wildlife Refuge, San Patricio Co., TX, USA, and at Hobcaw Barony, Georgetown Co., SC, USA. There is considerable geographic variation in signal duration and signal period (time from onset of signal to onset of following signal) in *H. cinerea* (Asquith et al. 1988; Höbel and Gerhardt 2003). Average (±SD) signal duration was 128±17 ms in the TX site and 161±19 ms in the SC site. Average (±SD) signal period was 551±56 ms (coefficient of variation (CV), 0.25± 0.06) in the TX site and 759±160 ms (CV, 0.21±0.04) in the SC site. Consequently, I always tested for site effects in signal timing behavior, but if none was detected I present data using the combined data set.

Recording equipment and procedure

I selected semi-isolated dyads of vocally interacting males and recorded their signals for 15 min ($N=3$ dyads in TX, $N=6$ dyads in SC). The minimum distance between recorded male dyads to other males signaling at the breeding pond was at least 10 m. The mean (±SD) inter-male distance between vocally interacting males was similar at both recording sites (4.3±0.4 m (TX) and 4.6±0.6 m (SC); $t=2.02$, $P=0.73$).

I used a Sony TC-D5M stereo cassette recorder and two Audio-Technica US ATR55 microphones mounted on tripods to record the males and later digitized the recordings at a sampling rate of 22.5 kHz using CoolEdit96 software (Syntrillium Corp.). I recorded an average of 946 signals per male (range, 165–1774) in the course of each 15-min recording period. After each recording, I measured the snout–vent length (SVL) of the males, the distance between them, and the air temperature (with a Miller and Weber cloacal thermometer).

Signal timing and signal attractiveness

To describe relative signal timing, I measured the signal delay (time from onset of signal of one male to the onset of the other male's signal) and the signal period (time between onsets of consecutive signals of the same male) of every recorded signal. From these absolute signal timing values (in milliseconds), I calculated the phase angles (in angular degrees) at

which signals were placed. Phase angles take the signal period of one male as a reference point and relate the timing of the other male's signal to this reference (Klump and Gerhardt 1992). Phase angles thus effectively correct for variation within and between males in the length of signal periods. Although it is not known whether females use phase angle information when making mate choice decision, I used this measure because it allows for direct comparison of signal timing behavior between males and between sites.

Phase angles can range from 0° to 360°, with values around 0° and 360° indicating signal overlap and values around 180° indicating signal alternation. I plotted phase angle histograms (bin width 10°) for visual inspection of the signal timing pattern of each male. I also used this phase angle data to obtain two descriptions of male signal timing behavior. First, I obtained a relative-timing-based description of signal timing, which is purely an account of male signaling behavior and does not consider the attractiveness ranking of a particular signal timing position. For this, I grouped the data of each male into three categories and categorized phase angles between 1° and 159° as lagging signals, phase angles between 160° and 200° as alternating signals, and phase angles between 201° and 359° as leading signals. For each male, I calculated the proportion of signals given in each signal timing format (lagging, alternating, leading), as well as the modal phase angle, i.e., the phase angle at which most of a male's signals were placed in each of the three signal timing formats. To determine how consistently males were signaling in a particular format, I also counted the number of consecutive signal periods over which each male signaled in one format (i.e., leading) before switching to another (i.e., alternating or lagging). Second, I obtained a signal-attractiveness-based description of male signal timing by incorporating information about the duration of the forbidden interval of *H. cinerea* females into the analysis (Höbel and Gerhardt 2007). For this, I grouped the data of each male into three categories and categorized signals that were leading overlapping as attractive (Fig. 1a, top trace), signals outside the forbidden interval (irrespective of leading or lagging position) as neutral (Fig. 1b, c), and signals that were lagging overlapped as unattractive (Fig. 1a, bottom trace). For each male, I calculated the proportion of signals given in each category (unattractive, neutral, attractive). Finally, I compared whether the proportion of leading signals given by a male was a good predictor for the proportion of attractive ones and whether the proportion of attractive signals was correlated with the proportion of unattractive ones.

Arhythmic signaling and signal overlap

Most males did not signal continuously for the duration of the 15-min recording period. These gaps in rhythmic signaling activity showed two patterns, which I termed "breaks" and "skips." Breaks are longer periods of silence (> 5 signal periods) between signaling bouts (see Fig. 1d). To evaluate the effect that taking a break and then starting a new signaling bout had on relative signal timing, I calculated the phase angle of the first signal of the focal male after a break (this refers to the first signal for which an angle can be calculated, i.e., the fist signal that falls between two signals of the other male). I also calculated the phase angles of the first ten signals of the focal male after a break and I recorded which male initiated each new signaling bout.

Skips are short periods of silence during which one male (A) skips a signal while the other (B) produces two signals before the first male signals again (i.e., A–B—B–A) (Fig. 1e). When investigating skips, I first evaluated the relative frequency of skips by calculating the proportion of signal exchanges that were of the rhythmic type (A–B–A–B) and those that involved skips (A–B—B–A). Then, I evaluated which male initiated a skip by comparing the signal periods of both involved males before, during, and after a skip. If the signal period of male A is longer during the skip than before and after the skip (while the signal period of male B is constant), this would indicate that male A initiated the skip by prolonging its signal period such that two signals of male B are produced before male A signals again. If, however, the signal period of male B is shorter during the skip than before and after the skip (while the signal period of male A is constant), this would indicate that male B initiated the skip by shortening its signal period and producing two signals before male A signals again.

Next, I evaluated the effect of skips on the outcome of signaling interactions. I focused on two aspects: (1) skip-related changes in signal production rate and (2) skip-related changes in relative signal timing. Because skips resulted from the skipping male prolonging its signal period (see results; Fig. 4a), frequent skips should be associated with a reduction in signal production rate. I therefore counted the number of signals that each male gave during the course of the 15-min recording periods, as well as the proportion of skips, and compared the resulting signal rates between the two interacting males in each dyad. To evaluate skip-related changes in relative signal timing, I compared the phase angles of signals just before and just after a skip. For this analysis, I compared whether skipping resulted in predictable changes in signal timing format (e.g., from lagging to leading), and I also compared whether the frequency of signal overlap increased during skips. Relative signal timing during skips can take three forms: either no signal overlap occurs (Fig. 1e, lower central panel) or signals do overlap. In case of signal overlap, two arrangements are possible: The first signal of male A overlaps the first signal of male B (Fig. 1e, lower left panel), in which case the signal of male A would be attractive (leading

overlapping) and the one of male B unattractive (lagging overlapped), or the second signal of male B overlaps the second signal of male A (Fig. 1e, lower right panel), in which case the signal of male B would be attractive and the one of male A unattractive. Whenever signal overlap occurred during a skip, I also evaluated which male (the skipper or the double-signaler) gave the attractively timed signal.

Effects of body size and signal period on signal timing

I evaluated the effect of body size and modal signal period on four signal timing parameters: the proportion of leading signals, the proportion of attractive signals, the proportion of unattractive signals, and the proportion of skips. I looked at the effect of the absolute male trait value on signal timing, and I also evaluated the effect of relative differences in trait values by calculating the magnitude of the difference in size or signal period between pairs of interacting males. For statistical analysis, I normalized the relative differences between interacting males by dividing the difference in size and signal period, respectively, by the within-populations mean for each of the two recorded populations. When graphing signal timing in relation to relative differences in size or signal period, I used the signal timing data from the larger male and the faster signaling one, respectively.

Statistical analysis

Proportional data were arcsine-transformed before statistical analysis. Because I had collected data in geographically separate sites, I used a two-step approach when calculating test statistics. First, I calculated a model that included site as a factor, as well as site as an interaction term. When the interaction term was not significant, I removed it to increase power and repeated the calculation. I used the statistical package JMP 7 to calculate test statistics. Descriptive statistics are presented as mean ± one standard deviation; whenever appropriate, I additionally present the range of observed values in parentheses. Whenever populations did not differ significantly, I combined the data before calculating means. For some comparisons, I complement the analysis by providing effect sizes. I calculated effect size with the following equation, which is applicable for any F with df=1 in the numerator (Rosenthal 1991; page 19):

$$r = \sqrt{(F/F + \mathrm{df}_{\mathrm{error}})}$$

This r is bounded between 0 and 1 and allows expressing effect sizes in standardized categories of small ($r<0.20$), medium ($0.2<r<0.5$), and large ($r>0.5$) (Cohen 1988).

Results

Rhythmic signaling, where one signal of male A was followed by one signal of male B (Fig. 1a–c), was the predominant form of signal interaction in H. cinerea and accounted for 91.8±5.6% of all recorded signals. Skips and breaks were much less frequent (see below).

Most signals (90.4±6.3%; range, 76.7–98.4%) fell outside the forbidden interval and were therefore in selectively neutral position. Only 5.3±3.7% of all signals were in attractive position, and 4.3±2.9% were in unattractive position. Although the average proportion of overlapped signals was low, there was considerable variation in the proportion of attractive (range, 0.5–14.2%) and unattractive (range, 0.3–9.9%) signals produced by individual males.

Males that gave a higher proportion of leading signals gave a lower proportion of attractive ones (Fig. 2a; effect of percent of leading signals: $F_{1,15}=8.022$, $P=0.013$; effect of site: $F_{1,15}=1.03$, $P=0.33$) as well as a lower proportion of unattractive ones (effect of percent of leading signals: $F_{1,15}=6.512$, $P=0.022$; effect of site: $F_{1,15}=0.97$, $P=0.34$). Males that gave a higher proportion of leading signals did not produce an absolute higher number of attractive signals (effect of percent of leading signals: $F_{1,15}=2.33$, $P=0.15$; effect of site: $F_{1,15}=1.76$, $P=0.20$), and having an overall higher proportion of attractive signals was not an artifact of signaling more (effect of number of signals: $F_{1,15}=0.624$, $P=0.44$; effect of site: $F_{1,15}=0.08$, $P=0.78$). Further, males that gave a higher proportion of attractive signals also gave a higher proportion of unattractive ones (Fig. 2b; effect of percent of attractive signals: $F_{1,15}=1253.95$, $P<0.0001$; effect of site: $F_{1,15}=0.006$, $P=0.94$).

Patterns of signal timing during signal interactions

The signal timing behavior of naturally interacting males was characterized by a combination of consistent as well as highly variable features. Among the consistent features
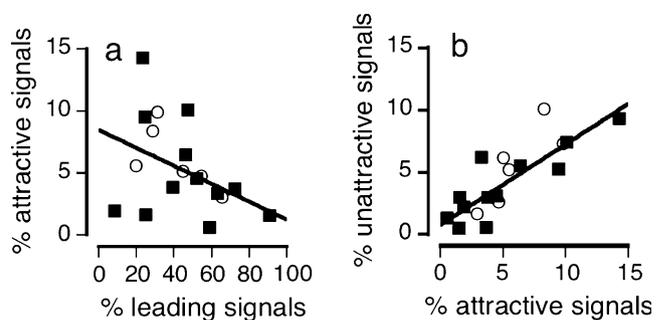

Fig. 2 Scatterplots showing the relationship between **a** the proportion of leading and attractive signals produced by each male and **b** the proportion of attractive and unattractive signals produced by each male. *Filled squares* denote data from South Carolina; *open circles* denote data from Texas

were the phase angle values at which males placed their signals: there was a preferred phase angle for signaling in leading (253±34°), alternating (180±11°), and lagging format (109±33°). In contrast to the preferred phase angles, there was substantial between-male variation in the proportion of signals given in a particular signal timing format. The pattern varied from both interacting males producing about equal numbers of signals in leading and lagging format (33% of recorded interactions; Fig. 3a) to interactions in which one male gave mainly leading signals while the other male produced mainly lagging ones (11% of recorded interactions; Fig. 3b). Most interactions showed an intermediate pattern, with one male giving somewhat more signals in leading format than the other male (56% of recorded interactions; Fig. 3c).

Another surprisingly dynamic and variable feature was the rate at which males changed between signal timing formats (leading, alternating, or lagging). On average, signal timing format changed every 3.5±7 signal periods (Fig. 3d), suggesting that males do not fall into a set signaling pattern but rather constantly readjust their signal timing behavior to each other. Again, there was considerable variation in how consistently males adhered to one signal timing format before switching to another (range, 1–132 signal periods).

Arhythmic signaling and its effect on signal interactions

Taking a break from signaling and partitioning signaling into bouts was relatively infrequent. Although breaks occurred in three of three interactions recorded in Texas and three of six interactions recorded in South Carolina, the number of breaks in each 15-min recording period was generally small (average, 3±2.6; range, 1–8).

Initiating a new signaling bout did not result in signal timing at specific phase angles; the average phase angle of the first signal starting a new signaling bout was 179±67° (range, 40–342°). Also, signal interactions were highly variable from the start. The phase angles of the first ten signals after the initiation of a new signaling bout (average, 176±81°; range, 6–358°) were as variable as the phase angles during long-term interactions (180±28°; range, 1–358°), and on average males switched signaling format (leading, alternating, or lagging) 2.8±1.5 times (range, 0–5) during the first ten signal periods.

Skips were more frequent than breaks but much less frequent than rhythmic signal exchanges. On average, 7.7±6.7% of all signal exchanges were skips. Again, there was substantial variation in the proportion of skips produced by individual males (range, 0.7–25.1%).

Skips were initiated by the male that skipped the signal (i.e., male A in an A–B—B–A interaction). During the skip, the signal period of the skipping male (here A) was on average 43±20% longer than his pre-skip period, while the signal period of the double-signaling male (here B) was 5±4% shorter than his pre-skip period (Table 1; Fig. 4). This suggests that skips result from the skipping male prolonging his signal period, not from the other male placing two signals quickly within the signal period of a neighbor's signals. Note also that the skipped signal period was not twice as long as the pre-skip one, i.e., the skipping male did not simply drop out for one signaling cycle.

Fig. 3 Phase angles of *H. cinerea* signals during signal interactions. **a** Phase angle histograms of the dyad in which both males produced an approximately equal proportion of leading and lagging signals. **b** Phase angle histograms in which one male (here B) consistently produces leading signals, while the other one mainly signaled in follower position. **c** Phase angle histograms in which one male gives somewhat more signals in one signal timing format (leading or lagging, respectively) than the other male. Shown is the proportion of signals given in each of 36 consecutive 10° bins, bin one starting at the onset of the signal given by the male whom the focal male was interacting with. **d** Phase angles of 100 consecutive signals of a representative male
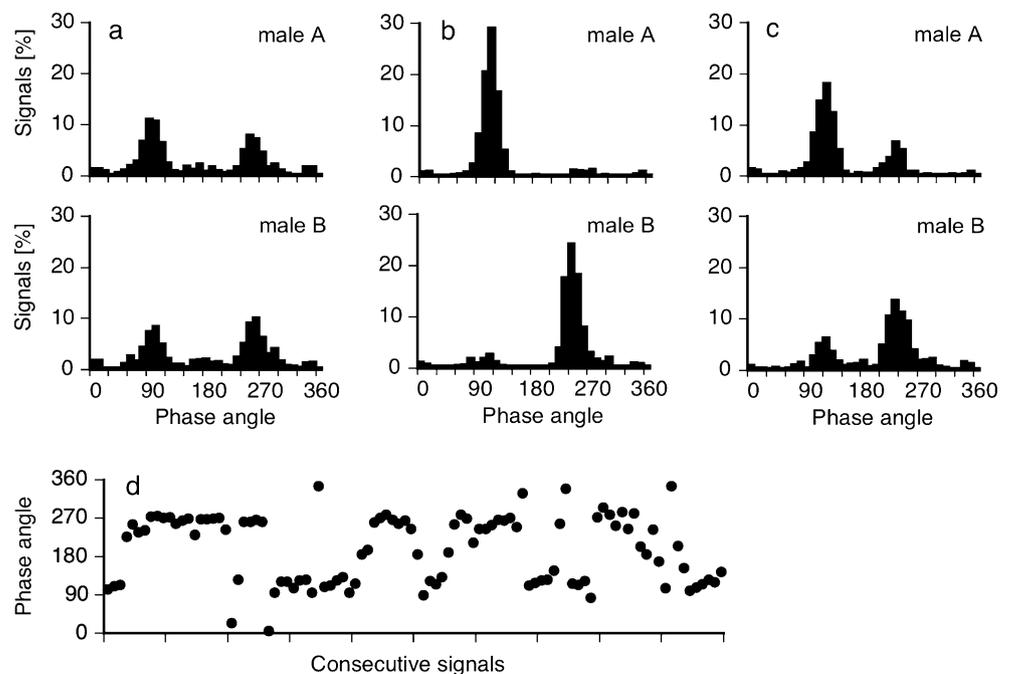
Table 1 Results of ANOVAs testing whether signal periods of skipping males and double-signaling males differed before, during, and after a skip (see Fig. 4a, b)

	Source of variation	df	F	P
Skipping	Site	1, 17.7	9.5147	*0.0065*
	Position (before, during, after)	2, 3,340	218.3837	*<0.0001*
	Interaction (site × position)	2, 3,340	4.0650	*0.0172*
	Individual	16, 3,340	50.7897	*<0.0001*
Double signaling	Site	1, 16.4	4.6141	*0.0469*
	Position (before, during, after)	2, 3,366	25.9494	*<0.0001*
	Interaction (site × position)	2, 3,366	3.6983	*0.0249*
	Individual	16, 3,366	173.9911	*<0.00001*

P values with significant difference were set in italics

Skips lead to a reduction of the number of signals produced over time. In six of the nine recorded dyads, both males skipped at similar rates so that the resulting difference in signal rate was relatively small (0.2–5.8%). In three dyads, however, the signal rate differences amounted to 11–16%.

Skips were associated with changes in signal timing format. Individual, signal position (before/after), site, and the interaction between position and site were entered as variables in a general linear model to examine their effect on signal timing format. All variables except site were significant: individual $F_{16, 6,078}=5.422$, $P<0.0001$; signal position $F_{1, 6,078}=748.9$, $P<0.0001$; position × site $F_{1, 6,078}=13.74$, $P=0.0002$; site $F_{1, 39.6}=1.065$, $P=0.31$. Pre-skip phase angles of the skipping male were largely in leading format, while post-skip phase angles were in lagging format (Fig. 5). More importantly, overlapped signals were dispro-

portionately frequent during skips. Although only a fraction of the observed signal interactions were skips (7.7±6.7%), the majority of overlapped signals recorded for each male occurred during skips (76.3±17.7%). Of the overlapped signals that occurred during skips, 49.2±31.4% were attractively timed signals by the skipping male (Fig. 1e, lower left panel), while 50.8±31.3% were attractive signals by the double-signaling male (Fig. 1e-lower right panel).

Influence of body size on signal timing

Absolute male size was not associated with the proportion of attractive signals, the proportion of unattractive signals, or the proportion of skips (Table 2). Overall, larger males did produce a larger proportion of leading signals (effect size of medium magnitude, $r=0.46$), although this term was not significant in the ANOVA (Table 2). When the comparison took differences in body size between interacting males in a dyad into account, then more effects of body size on signal timing were found. The larger the difference between interacting males, the more leading signals were produced by the larger male ($r=0.798$; Table 2; Fig. 6a). The larger male in an interactions also produced a smaller proportion of attractive signals (effect size of large magnitude; $r=0.656$), although this term was not significant in the
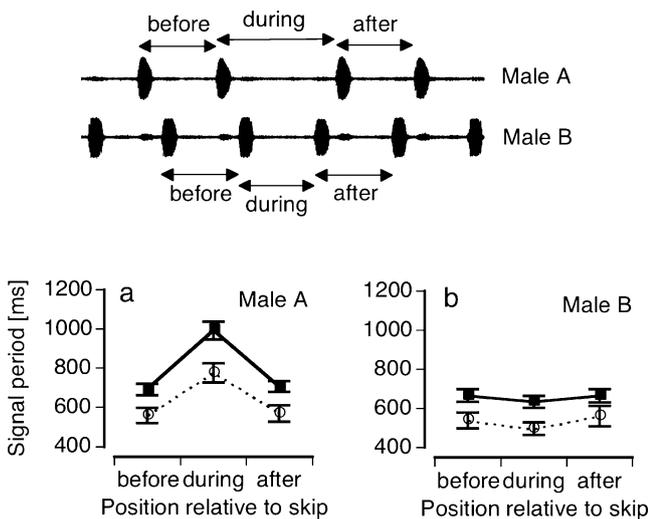

Fig. 4 Average signal periods before, during, and after skips. **a** Signal periods of skipping males increased during skips. **b** Signal periods of double-signaling males decreased. *Filled squares* indicate data from South Carolina; *open circles* indicate data from Texas

Fig. 5 Mean phase angles of signals given by the skipping male before and after a skip. *Filled squares* denotes data from South Carolina; *open circles* denote data from Texas
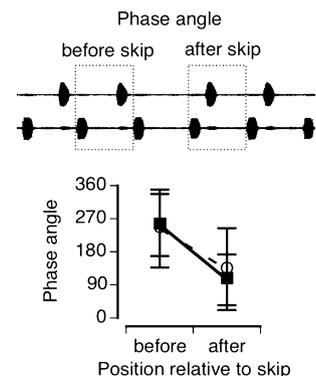
Table 2 Results of ANOVAs testing whether body size (top rows) and differences in body size (bottom rows) affect the signal timing behavior of male *H. cinerea* (see Fig. 6)

	df	Leading signals		Attractive signals		Unattractive signals		Skips	
		F	P	F	P	F	P	F	P
Effect of absolute body size									
Site	1, 15	0.050	0.826	0.578	0.45	0.579	0.459	0. 551	0.469
SVL	1, 15	4.034	0.063	0.674	0.425	0.591	0.454	0.041	0.842
Effect of differences in body size									
Site	1, 6	1.168	0.321	0.130	0.731	0.278	0.617	0.116	0.745
SVL difference	1, 6	*10.542*	*0.018*	4.521	0.078	2.004	0.207	0.245	0.638

P values with significant difference were set in italics

ANOVA (Table 2; Fig. 6b). Size difference was not associated with the proportion of unattractive signals (Table 2; Fig. 6c) or with the proportion of skips (Table 2; Fig. 6d).

In all of the six interactions in which male signaling was partitioned into discrete signaling bouts, it was the larger male that more frequently (75–100%) initiated a new bout (in the remaining three interactions, one or both males signaled continuously, so no data on bout initiation could be obtained).

Influence of signal period on signal timing

Absolute signal period did not affect signal timing (Table 3). The magnitude of the difference between signal periods of

Fig. 6 Scatterplots showing the relationship between the difference in body size between interacting males in a dyad and signal timing. The proportion of leading signals indicated is the one given by the larger male in each dyad. **a** The larger the size difference, the more leading signals were produced by the larger male. **b** The larger the size difference, the fewer attractive signals were produced by the larger male, although not significantly so. **c** Size difference was not associated with the proportion of unattractive signals. **d** Size difference was not associated with the proportion of skips. *Filled squares* denote data from South Carolina; *open circles* denote data from Texas

interacting males, on the other hand, affected several signal timing parameters, and the effect size was of large magnitude (see below). The faster signaling male in a dyad produced fewer leading signals if the signal period difference between the males was large (r=0.873; Table 3; Fig. 7a). Also, the faster signaling male in a dyad produced more attractive (r=0.947; Table 3; Fig. 7b) as well as more unattractive signals (r=0.884; Table 3; Fig. 7c). The proportion of skips was not influenced by the difference in signal period between pairs of interacting males (Table 3; Fig. 7d).

Discussion

This study documents substantial variation in male signal timing behavior. The majority of variation consists of signal timing positions that do not influence male attractiveness. For example, variation in the proportion of leading and lagging signals produced by individual males (Fig. 3a-c) or frequent changes in signal timing format (Fig. 3d) was very prevalent in *H. cinerea*, but since it involves mainly signals outside the forbidden interval it should not influence female choice. Frequent switches between males occupying the leader or follower role during signaling interactions seem to be common in group-signaling animals and have been reported for other anurans (Schneider et al. 1988; Bosch and Márquez 2001; Grafe 2003) as well as insects (Walker 1969; Greenfield and Roizen 1993; Hartbauer et al. 2005). The present study also documents variation in signal timing behavior that should influence male attractiveness because it involves signals within the forbidden interval, in particular leading overlapping (attractive) and lagging overlapped (unattractive) signals. The number of signals in this category is much smaller, comprising only 10% of all signals given by male *H. cinerea* (4.3% lagging overlapped; 5.3% leading overlapping). Similarly, low values for overlapped signals seem to be the rule for group-displaying species with non-synchronous chorus structure (Schwartz 1993; Grafe 1996; Greenfield and Snedden 2003). This overall pattern of high variability in the aspect of signal timing behavior that females do not
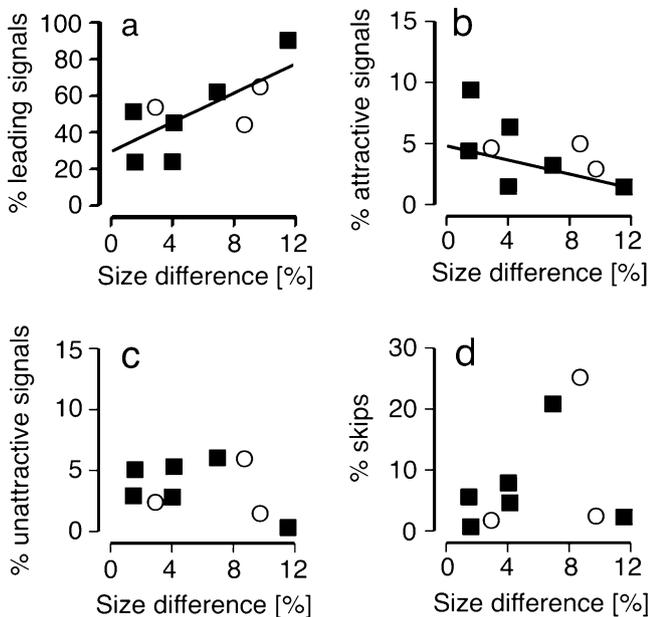
Table 3 Results of ANOVAs testing whether signal period (top rows) and differences in signal period (bottom rows) affect the signal timing behavior of male *H. cinerea* (see Fig. 7)

	df	Leading signals		Attractive signals		Unattractive signals		Skips	
		F	P	F	P	F	P	F	P
Effect of absolute signal period									
Site	1, 15	0. 240	0.879	0.955	0.344	0.843	0.373	0.657	0.430
Signal period	1, 15	0.160	0.695	0.013	0.911	0.001	0.981	0.143	0.710
Effect of differences in signal period									
Site	1, 6	0.282	0.615	0.489	0.511	0.067	0.617	0.9512	0.367
Signal period difference	1, 6	*19.132*	*0.005*	*52.526*	*0.001*	*21.496*	*0.004*	0.098	0.765

P values with significant difference were set in italics

attend to, combined with low variability in aspects that they do, suggests that sexual selection by female choice has played a major role in shaping the signal timing behavior of males in this and other group-displaying species.

What role does current variation in signal timing behavior play in determining male attractiveness? Although the average proportion of overlapped signals was low, the range of variation between individual males was not insubstantial, ranging from 0.5% to 14.2% for attractive

Fig. 7 Scatterplots showing the relationship between the difference in signal period between interacting males in a dyad and signal timing. The proportion of leading signals indicated is the one given by the male with the shorter signal period (i.e., faster signaling male). **a** The larger the difference in signal period, the fewer leading signals were produced by the faster signaling male. **b** The larger the difference in signal period, the more attractive signals were produced by the faster signaling male. **c** The larger the difference in signal period, the more unattractive signals were produced by the faster signaling male. **d** Signal period difference was not associated with the proportion of skips. *Filled squares* denote data from South Carolina; *open circles* denote data from Texas

signals and from 0.3% to 9.9% for unattractive ones. Whether variation of this magnitude is sufficient to result in a significant preference for the male producing more attractively timed signals is difficult to determine at present because playback trials testing signal timing preferences generally present the alternatives in a way in which one alternative is maintained in attractive position for every stimulus presentation (i.e., 100% attractive signal timing) (Grafe 1996; Greenfield et al. 1997; Bosch and Márquez 2002; Höbel and Gerhardt 2007). Given the overall relatively low rates of signal overlap found in this and other studies (Schwartz 1993; Grafe 1996; Greenfield and Snedden 2003), the standard experimental protocol for testing signal timing preferences grossly overestimates the degree of variation in attractive signal timing that females are likely to encounter when making mate choice decisions in nature. Consequently, it would be premature to conclude that any male that produces more attractively timed signals will also have higher mating success. To tackle this question, it will be important for future studies to gather information on the minimum proportion of attractive signals necessary to result in a significant preference in females of a given species. In addition, studies looking at attractive signal timing should not only report that a given male produced more attractively timed signals but rather provide data on the relative proportion of this signal timing category for each male. Without this information, the real live impact of signal timing behavior on male attractiveness will remain elusive.
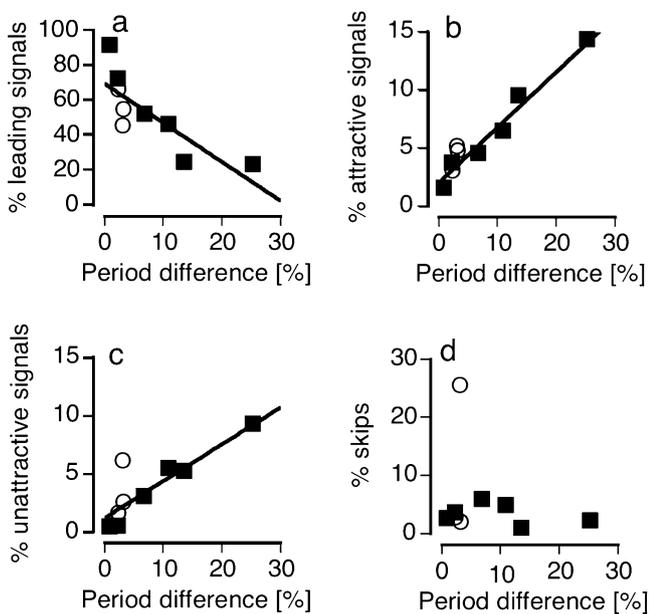
By having simultaneously assessed a range of signal timing parameters at both population and signaling dyad level, the present study also highlights the importance of choosing the correct parameters and levels of comparison when evaluating signal timing behavior. First, the scale of comparison matters. In *H. cinerea*, overall larger size or shorter signaling period was generally not associated with signal timing, but when the comparison focused on relative differences between dyads of interacting males a much stronger link between size or signal period and signal

timing became apparent (Tables 2 and 3; Figs. 6 and 7). Second, if the goal of a study is to describe variation in signal timing or the relationship between male traits (e.g., signal feature, size, condition) and signal timing, then a range of signal timing parameters (e.g., leading signal, attractive signal) may be equally appropriate to score. However, if the goal is to assess the importance of signal timing behavior for male attractiveness, then scoring the proportion of leading signals may result in equivocal conclusions about the attractiveness ranking of males. In *H. cinerea*, not only was the proportion of leading signals given by a male not a good predictor of the proportion of attractive ones, the pattern was in fact reversed (Fig. 2a). Moreover, *H. cinerea* males that produced more signals in attractive position also produced more signals in unattractive position (Fig. 2b). Unless females weigh attractive signals stronger than the unattractive ones, this may net balance the effect of signal timing on male attractiveness. In other species, the production of attractive and unattractive signals may be independent of each other, but it will be crucial to verify this before discussing fitness benefits of attractive signal timing. Scoring the proportion of attractively timed signals without investigating the potential for a concurrent increase in the proportion of unattractively timed ones may otherwise lead to erroneous conclusions about a male's signal timing-based attractiveness ranking.

Furthermore, the observation that *H. cinerea* males that produce more signals in attractive position also produce more signals in unattractive position is interesting because it suggests that males may be using different signal timing strategies: (1) accurate timers, those who take greater care in placing their signals outside the forbidden interval, thus producing mainly signals in neutral position; and (2) inaccurate timers, those who are less careful in placing their signals outside the forbidden interval, thus producing a larger proportion of signals not only in attractive but also in unattractive position. If similar proportions of attractive and unattractive signals cancel each other out, both strategies should be equally attractive. However, if females weigh attractively placed signals stronger than the unattractive ones, inaccurate timers may gain an edge because they will have more attractively timed signals. If, on the other hand, females value display strategies that allow them to accurately evaluate signal features, then accurate timers may benefit because they will have more signals in neutral positions whose features can be evaluated without interference from overlapping signals.

Effect of signal features and signal timing on male attractiveness

When comparing across all recorded *H. cinerea* males, body size and signal period were not correlated with signal timing parameters (Tables 2 and 3). A similar lack of association between body size or condition and signal timing has been reported for other anurans (Bosch and Márquez 2001; Richardson et al. 2008), but other studies have found that males with shorter signal periods produce more signals in leading/attractive timing positions (Hartbauer et al. 2005; Richardson et al. 2008). However, this lack of association may be an artifact of the scale of comparison.

A male's neighbor is probably his strongest competitor because the close proximity allows females to directly compare their signaling behavior. In this respect, it is interesting that, when the comparison was focused on dyads of vocally interacting males, differences in size/signal period were more frequently associated with differences in signal timing. Yet because correlations were sometimes positive and sometimes negative, an association will not always result in an attractiveness-increasing combination of traits. For example, large differences in signal period between males in a dyad resulted in more attractively timed signals by the faster signaling male (Fig. 7b), which should increase overall attractiveness, while the concurrent production of more unattractively timed signals (Fig. 7c) should decrease it. There was also a trend for large differences in size between males in a dyad to result in fewer attractively timed signals by the larger male (Table 2; Fig. 6b). Consequently, three traits shown to be important for female choice in this species (Gerhardt 1987; Höbel and Gerhardt 2007) can combine in ways that may increase as well as decrease male attractiveness. Predicting the outcome of these trait combinations is not straightforward because preferences for signal feature and signal timing traits may interact with each other (e.g., Dyson and Passmore 1988; Smith and Roberts 2003; Berg and Greenfield 2005; Höbel 2010) and because the patterns of interaction may not be equally strong between traits. For example, in *H. cinerea*, the preference for low-frequency signals (associated with large body size) outweighs preferences for leading signals, but leader preference is somewhat stronger than the preference for short signal period (Höbel 2010).

Arhythmic signaling and its effect on signal interactions

Much of the observed variation in signal timing occurred during periods of arhythmic signaling, arguing that breaks and skips might be worthwhile focal points for future studies looking at the proximate mechanisms of signal timing. The importance of breaks and skips for male attractiveness and sexual selection is less clear. First, the majority of signal overlap occurred during skips. However, this included both signals that were attractive because of the overlap as well as those that were unattractive, and the production of attractive and unattractive signals was highly correlated. As outlined above, further research is needed to

determine whether this will result in a net balancing of overall attractiveness. Second, breaks and skips were generally associated with changes in signal timing format (e.g., leading to alternating), but these changes do not necessarily translate into changes of the attractiveness ranking of the involved males. For breaks, I hypothesized that starting a new signaling bout is a strategy to join the chorus in a more attractive signal position. This was not the case, as first signals after bout initiation were placed at diverse phase angles (range, 40–342°). For skips, the expectation is that the male initiating the skip should benefit from doing so, but the data did not support this prediction either. Although signals before and after a skip were placed at different phase angles, signals after a skip were not in more attractive positions (Fig. 5). Third, because a male produces one fewer signal every time he skips and high signal repetition rate is almost universally preferred by females (Ryan and Keddy-Hector 1992; Gerhardt and Huber 2002; Greenfield 2002), skips should reduce the male's attractiveness. Present study documents a skip-related reduction in signal production rate, but in none of the recorded dyads did the difference exceed 16% (calculated over 15 min). Whether signal rate differences of this magnitude affect female choice may ultimately depend on the time that females take to make mate choice decision. Assessment time duration is unknown for *H. cinerea* females, but females of other North American treefrog species wait approximately 2 min before choosing and approaching a mate (Schwartz et al. 2004). If such short assessment times are the rule, signal skipping may not yield average signal rate differences of a magnitude capable of affecting female choice. On the other hand, short assessment times combined with stochastic effects may inflate signal rate differences, for example, if a female approaches and chooses a mate during a time in which a normally rhythmically signaling male skips an unusually large number of times.

In conclusion, breaks and skips do change the signal timing format in which males signal, and they concentrate a disproportionate amount of the signal overlap occurring during signaling interactions. However, their influence on a male's attractiveness, if there is one, may come less from direct effects of attractive signal timing and more from indirect effects through related changes in signal production rate.

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