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# Are leaders good mates? A study of call timing and male quality in a chorus situation

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Within insect and anuran choruses, females are exposed to many male sexual signals overlapping in time. In many species, male call timing influences mate choice, with females preferentially orienting towards males that succeed in calling just before their opponents. The mechanisms through which males actively regulate their call timing to achieve this have been the focus of much interest. However, no study has addressed the problem of the reliability of call leadership as an indicator of male quality, which is crucial to females, since basing their choice of mate on an unreliable criterion could lead them to engage in suboptimal matings, with detrimental effects to their fitness. Addressing this question in the European treefrog, *Hyla arborea*, we first ascertained that females preferentially orient towards the first of two identical overlapping conspecific calls. We then found that within experimental choruses the males with the highest proportion of leading calls had significantly higher total calling times, a call characteristic encompassing two indicators of signal costliness: call duration and call rate. Although we failed to find a link between call leadership and male body condition, this study provides novel evidence that female preference for leading calls leads to the selection of the males with the highest energetic investment in their sexual display. The handicap principle predicts that this form of female preference should increase the fitness of females by guaranteeing that they select high-quality mates.

**Keywords:** acoustic communication; anuran; call timing; chorus; European treefrog; *Hyla arborea*; mate choice; sexual selection

Choosing a mate is a crucial task, as it is a key determinant of the fitness of future offspring. Direct assessment of mate quality is often impossible, and therefore the choosing sex, most often the females, relies on the other sex, typically the males, to advertise their quality as potential mates through sexual signals. The accuracy of an individual's choice of mate thus rests on the honesty of these signals. Communication theory states that to be reliable, sexual signals must carry costs, and to meet such costs the signaller must have an adequate phenotypic quality. Thus, by comparing costly quality-dependent components of male advertisement signals, females are predicted to select high-quality mates since (1) attractive sexual signals are energetically costly to produce and

maintain, and (2) such costs are differentially higher for low-quality males than for high-quality males (Zahavi 1975). Much work has therefore concentrated on male signal content, the cost of the signal, its dependence on male phenotypic quality, and its role in intersexual selection. Research over the past few decades has brought to light a wealth of evidence that females use these costly quality-dependent signal components in mate choice (Andersson 1994). In many cases such forms of female preference lead to access to high-quality male territorial resources, or to offspring with a higher fitness (e.g. Welch et al. 1998; Forsman & Hagman 2006).

In acoustic species, signal cost is determined by at least three main call characteristics: duration, amplitude and rate of calling (Prestwich 1994; Reinhold et al. 1998; McLister 2001; Robinson & Hall 2002). However, female mate choice is not a function of signal characteristics alone. In nature, particularly in chorusing species, females are often exposed to several males calling within close proximity of each other. As early as 1975, female choice

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in Pacific treefrogs, *Hyla regilla*, was shown to be affected by the relative timing of the calls of neighbouring males (Whitney & Krebs 1975). Since then, many studies conducted on chorusing insects and anurans have found a female preference for males producing leading calls, that is, the first of two or more spatially separated signals broadcast within a brief time interval (Whitney & Krebs 1975; Dyson & Passmore 1988a; Klump & Gerhardt 1992; Howard & Palmer 1995; Grafe 1996; Greenfield & Rand 2000; Bosch & Márquez 2000). In several species this preference has been shown experimentally to override limited differences in costly call components such as call duration or call amplitude (Dyson & Passmore 1988a, b; Greenfield & Roizen 1993; Grafe 1996).

Preference for leading calls could be the result of several mechanisms (reviewed in Andersson & Simmons 2006): (1) direct phenotypic effects, if male ability to produce leading calls reflects ability to provide immediate advantages, (2) indicator mechanisms ('good genes' or 'handicap mechanisms') if production of leading calls reflects broad genetic quality, or (3) female sensory bias if calls uttered within a brief interval of a leading call are harder to detect, localize or discriminate than the latter, as has been suggested by research on acoustic masking (reviewed in Litovsky et al. 1999). Under all three scenarios, males are predicted to engage in signal competition, whereby they strive to increase the proportion of attractive leading calls they make. However, while under the evolutionary scenarios (1) and (2) male production of leading calls used for mate choice is predicted to be costly and females are predicted to accrue direct or indirect benefits, situation (3) is open to two evolutionary developments first elaborated by West-Eberhard (1984). The evolution of novel male traits that exploit a pre-existing female preference for leading calls can indeed, if the exploitative trait is, or becomes through male competition, costly, lead to female choice of high-quality mates. However, if the production of leading calls is not a costly form of signalling, female preference for leading males is predicted to result in maladaptive mate choices, which could over time lead to an erosion of the species' fitness. Surprisingly, despite much interest in recent years in the mechanisms underlying female preference for leading calls, little or nothing is known of the reliability of male call leadership as a criterion for female mate choice, that is, is the production of such calls energetically costly? And does it reflect male phenotypic quality?

We addressed this problem in the case of the rhythmically calling European treefrog, *Hyla arborea*. In this species, female mate choice takes place in choruses with densities that can be extremely high, with records of several hundred males present simultaneously on a given breeding site (personal observations). Thus a high degree of call overlap is present in natural choruses of *H. arborea*, making it a likely candidate for female preference for leading calls. In addition, call production is extremely costly in this species, with calling metabolism being the highest measured so far in ectothermic vertebrates (Grafe & Thein 2001). Our aim was first to test, through a simple two-stimuli phonotaxis experiment, the existence of a female preference for

leading calls in *H. arborea*. We then determined, by staging and recording experimental four-male choruses, whether male call leadership is associated with an energetically costly form of signalling. Our third objective was to test whether call leadership reflects male phenotypic quality. To do this, we chose to investigate the relationship between male call leadership and an indicator of male phenotypic quality traditionally used: male body condition. The hypothesis tested is that, should call leadership prove to be a costly form of signalling, a preference for leading males should lead to the selection of males with good body condition, that is, males that carry a sufficient amount of energetic reserves to fuel their costly courtship behaviour.

## GENERAL METHODS

### Study Species

*Hyla arborea* is a treefrog (mean weight  $\pm$  SD =  $4.41 \pm 0.62$  g, Márquez et al. 2005) found throughout central and eastern Europe. During its prolonged breeding season, from mid-April to the end of May in our latitudes, the males congregate around ponds from which they call from dusk until around midnight in favourable meteorological conditions (no wind, minimal temperature:  $4.5^\circ\text{C}$ ; Friedl & Klump 2002). Males call in sequences, or bouts, of advertisement calls, with mean overall call rates  $\pm$  SD of  $2.33 \pm 0.95$  calls/s and within-bout call rates of  $7.15 \pm 0.73$  calls/s (personal observations of the study population). Individual calls are short ( $\bar{X} \pm$  SD =  $73.8 \pm 8.5$  ms), with a mean fundamental frequency  $\pm$  SD of  $953.1 \pm 79.1$  Hz (Castellano et al. 2002), and consist of several pulses ( $\bar{X} \pm$  SD =  $7.88 \pm 0.36$ , Friedl & Klump 2002). Temporal call parameters are temperature dependent, and dominant frequency is negatively correlated with male body weight (Friedl & Klump 2002).

### General Experimental Conditions

We conducted our study from mid-April to mid-May with the approval of the préfecture de L'Isère, France. From mid- to the end of April, 107 males and 20 gravid females were collected from two ponds in the vicinity of Lyon in the southeast of France. Throughout the experiment the males were housed individually in terraria ( $32.5 \times 17.5$  cm and 18.5 cm high) maintained at ambient temperature ( $20\text{--}25^\circ\text{C}$ ) and photoperiod, (15: 9 h light: dark), with constant access to fresh water, branches to perch on and small boxes to shelter in, and extra lighting provided daily between 1100 and 1800 hours, in synchrony with the local photo- and thermoperiod so as to avoid shifting the animals' daily activity rate. They were fed ad libitum with crickets, *Acheta domestica*. The females were tested 1–5 h after they were captured. All the animals were released without delay after testing, at the sites from which they were captured (1 day after capture for females, 6 days for males).

## EXPERIMENT 1: FEMALE PREFERENCE FOR LEADING CALLS

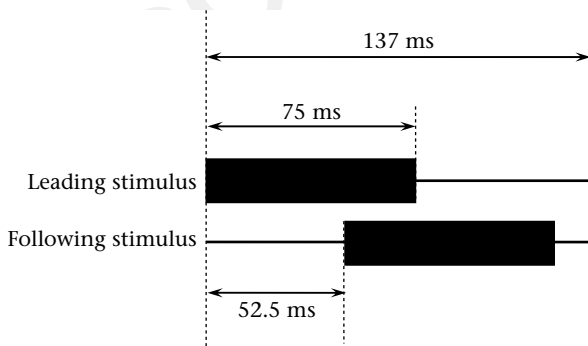
### Methods

To investigate female preference for leading calls, we conducted a phonotaxis experiment in which we presented females with two identical but spatially distinct overlapping male *H. arborea* calls. From our digital laboratory recordings of male *H. arborea* calls, we selected a 75 ms representative call (with a call fundamental frequency of 1009 Hz; sampling frequency: 16 kHz) which we used to construct a stereo track on which identical male calls were repeated every 137 ms with a 30% time overlap between the calls of the left and the right channels of the track (Fig. 1).

In the laboratory, two Monacor SP155X loudspeakers were placed on opposite sides of an arena (1.5 × 0.9 m and 1.0 m high). The walls of the arena were padded with insulating material to limit sound reverberation. Dim red lighting was used to facilitate observations while minimizing the effect of the lighting on the animal's behaviour. The temperature was maintained at 23 °C.

We broadcast the stimulus continuously from the two loudspeakers of the test chamber, which were connected to a notebook computer via an STA102 Stageline stereo amplifier to allow precise calibration of the broadcast sound level. Prior to each test, using a Voltcraft MT-8820 Sound Level Meter (C weighted, fast setting, tolerance ± 1.5 dB), we adjusted the peak sound pressure of calls for both loudspeakers to 75 dB at 1 m which is equivalent to values measured for natural calls at the same distance. The right and left channels of the stimulus track were randomly attributed to the two loudspeakers.

We collected 20 amplexed females in the field between 2000 and 2300 hours, and then transported them individually to the laboratory in small plastic boxes (14.5 × 10.5 cm and 6.0 cm high) containing a thin layer of water. Prior to testing, the females were maintained in these boxes in dark conditions for at least 15 min. The tests took place between 2400 and 0600 hours. At the beginning of each trial the female was placed under a cylinder of plastic mesh (diameter × height: 70 × 50 mm) in



**Figure 1.** Schematic representation of the acoustic two-channel stimulus which was looped and broadcast to females during phonotaxis tests. The calls of both channels had identical spectral and temporal properties.

the centre of the arena for 1 min while the stimuli were broadcast. The cylinder was then removed from a distance, and the female's movements were monitored. We scored a choice only when the female positioned herself on the membrane of one of the loudspeakers or within 10 cm of it and remained there for at least 15 s. If no choice was made within 10 min the trial was discontinued and not taken into account in the subsequent analysis. Each female was tested once. A two-tailed binomial test (with R 2.3.1 software, The R Foundation for statistical computing, Vienna, Austria) was used to determine whether there was a significant female preference for leading calls.

### Results

During the phonotaxis tests, two of the 20 tested females were unresponsive and were not taken into account in the analysis. The remaining 18 females positioned themselves on the membrane of one of the two loudspeakers or within 10 cm of them within a mean ± SD of 3.52 ± 3.01 min, and 15 of them showed a significant preference for the loudspeaker broadcasting leading calls (two-tailed 15/18 females chose predicted target, binomial test:  $P = 0.008$ ).

## EXPERIMENT 2: CALL LEADERSHIP AND COSTLINESS OF CALLING BEHAVIOUR

### Methods

The aim of this experiment was to determine whether male production of attractive leading calls in a chorus is associated with a costly form of signalling behaviour.

#### Constructing the four-male choruses

To examine male call interactions in a context closer to that of natural anuran choruses, we chose to study the phenomenon of call overlap in artificial four-male choruses rather than in male dyads as has more often been the case in past studies. In an attempt to create choruses with similar distributions of male quality, our first step was to rank all the males according to a measure of their call rate taken as an indicator of quality. To achieve this, over the 3 nights following their capture, between 2030 and 0200 hours, we made a 10 min digital recording for each of the 107 males. For each recording, a male was placed alone in a terrarium (32.5 × 17.5 cm and 18.5 cm high) in a dark room. A Beyer M300 TG microphone (frequency range 200–10 000 Hz ± 2 dB) was positioned 5 cm above the terrarium and connected to a notebook computer. A recording of a conspecific chorus (a 10 min selection of a recording of chorus noise of ca. 15 males, made on the study site in 2005, that lacked any distinct *H. arborea* calls and presented a close to constant amplitude) was broadcast with a Hitachi CX70 CD player at a low amplitude (50 dB) to stimulate the animal. We started recording the male as soon as he began calling (recording software: Cool Edit Pro, Syntrillium Software Corp., Phoenix, AZ, U.S.A; sampling frequency: 16 kHz).

Each male's call rate was then determined by a semi-automatic procedure. The amplitude envelope of each call

was calculated by using the analytical signal calculation described by Mbu Nyamsi et al. (1994) and digitally filtered (bandpass 0–70 Hz) by using the Syntana analytical package (Aubin 1994). These files were then analysed by a software program we developed to determine the number of calls produced per min during the time when the animal was actively calling, that is, between his first and last call. We considered this to be a measure of the animal's spontaneous call rate in conditions in which he was not actively interacting with another male.

The males were then sorted into three groups: 'slow' males with call rates below the first quartile of the call rate distribution, 'average' males with call rates ranging between the first and third quartile, and 'fast' males with call rates above the third quartile. Three days after capture, 18 artificial four-male choruses were then constructed by selecting, from among the calling males available, one 'fast' male, two 'average' males and one 'slow' male, thus homogenizing the distribution of male quality among the various choruses.

### *Chorus re-enactment*

To stage each chorus we placed each of the four selected males in a separate terrarium (32.5 × 17.5 cm and 18.5 cm high) in a dark room. The terraria were positioned in a circular array, 50 cm apart, to ensure that each male was able to hear and respond to the calls of his three neighbours. A recording of a natural *H. arborea* chorus (see description above) was broadcast with a Hitachi CX70 CD player at a low amplitude (50 dB) to stimulate the males' calling. A Sony ECM-T6 directional microphone was suspended inside each terrarium. The four microphone outputs were sent to a multichannel amplifier with a built-in multichannel analogue: digital converter (Alesis Multimix 8 Firewire, Alesis, Cumberland, RI, U. S. A.; sampling frequency: 44.1 kHz). The digitized output from the mixer was transferred to a notebook computer using Cubase Studio 4 software (Steinberg France, Le Kremlin-Bicêtre, France) and saved as a multichannel file. This set-up enabled us to obtain for each chorus four simultaneous 10 min recordings on four distinct audio channels. Because of the proximity of the four terraria, the microphones in a given terrarium did pick up the calls from males in the neighbouring terraria, but the difference in amplitude between these calls and those of the focal male enabled us to identify reliably on each audio file the calls that belonged to the focal male.

### *Quantifying male leadership: sound analysis*

The 72 sound files obtained after recording the 18 choruses were analysed following the procedure described previously to determine for each recorded individual: (1) the total number of calls, (2) individual call lengths, (3) the total number of calls that were overlapped by calls of other males, and (4) among these overlapped calls, the number of leading calls, that is, those that were first in a series of overlapping calls.

Within each chorus we were thus able to rank all four males according to the proportion of leading calls they produced (that is, the number of leading calls/the number

of overlapped calls, that is, the proportion of competitive interactions 'won' by relegating opponents' calls to an unattractive trailing position), which we used as a measure of their relative attractiveness (Greenfield & Roizen 1993).

### *Estimating male quality*

The objective of this experiment was to investigate the relationship between call leadership and male energetic investment in sexual display, which is determined mainly by call amplitude, length and rate. We were unable to measure male call amplitudes during the chorus recordings, because the males' positions and postures relative to the microphones changed as they moved within the terraria. We therefore chose to concentrate on the two temporal determinants of calling cost. To avoid multiple tests on correlated variables, we used total calling time, which encompasses both overall calling rate and individual call lengths (Prestwich 1994).

### *Statistical procedure*

During the chorus recordings, not all of the four males present called systematically, which could be caused by poor physical condition, or by other factors such as stress or lack of motivation. This uncertainty led us, after having ascertained that silent males did not differ in body condition from calling males (with a two-tailed exact Wilcoxon test for paired data on the indicators of body condition), to exclude these silent males from the data analysis. We thus obtained two choruses with two calling males, 11 choruses with three calling males and five choruses with four calling males. To be able to consider all 18 choruses independently of the number of calling males, within each chorus we compared the values of total calling time of the male producing the highest proportion of leading calls (referred to, for sake of simplicity, as the 'leading male') with those of the male producing the lowest proportion of leading calls (the 'following male'). Two-tailed exact Wilcoxon tests for paired data were used. For all the tests we used R 2.3.0 software. Call characteristic measurements are presented as mean ± SD.

## **Results**

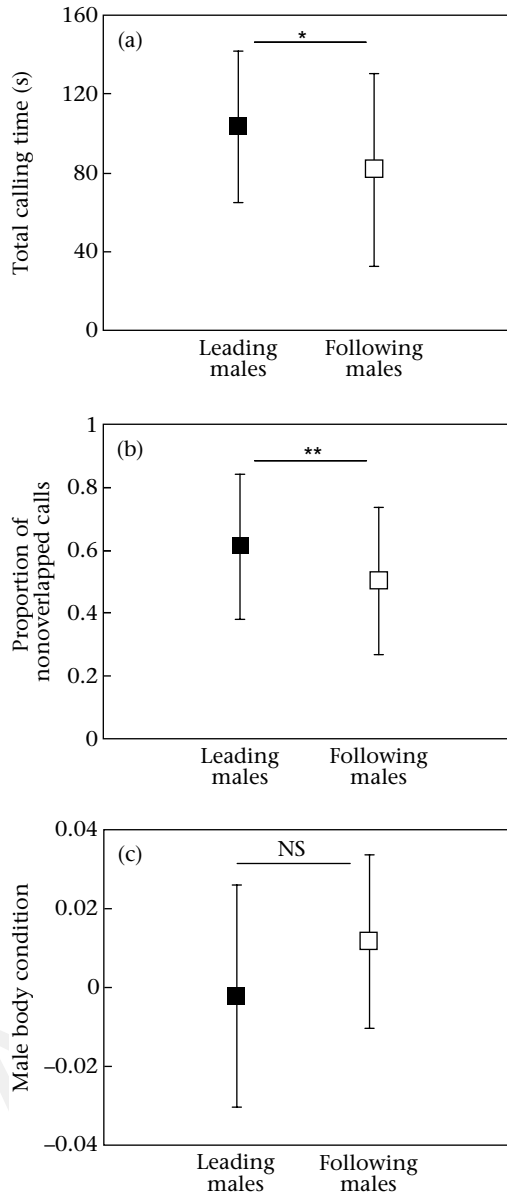
During the 18 chorus recordings, the calling males produced  $1411 \pm 673$  calls, with rates of  $8883 \pm 4202$  calls/h, (range 1298–34 252 calls/h). Individual calls had a mean length of  $73.8 \pm 8.5$  ms (51.2–98.6 ms).

The proportion of leading overlapped calls varied within the choruses: the most successful males, 'leading males', produced  $62.7 \pm 5.1\%$  of their overlapped calls in a leading position, whereas their least successful opponents, 'following males', produced only  $40.3 \pm 4.6\%$  of their overlapped calls in a leading position.

Leading males made a significantly higher total calling effort during the 10 min recordings:  $114.6 \pm 38.5$  s of calling time versus  $81.5 \pm 49.1$  s for following males (two-tailed Wilcoxon exact test for paired data:  $V = 131$ ,  $N = 18$ ,  $P = 0.048$ ; Fig. 2a). Thus male leadership, in terms of the proportion of leading overlapped calls, was

positively correlated with male energetic investment in sexual display behaviour.

Males with the greatest proportion of leading overlapped calls within a chorus also produced a significantly greater proportion of nonoverlapped calls than males with the lowest proportion of leading overlapped calls:  $61.2 \pm 0.2\%$  of their calls were not overlapped versus  $50.2 \pm 0.2\%$  for their least successful opponents (two-tailed Wilcoxon exact test for paired data:  $V = 156$ ,  $N = 18$ ,  $P = 0.001$ ; Fig. 2b).



**Figure 2.** Mean values  $\pm$  SD for the leading males (i.e. those producing the highest proportion of overlapped leading calls; closed squares) and the following males (those producing the lowest proportion of overlapped leading calls; open squares) in each of the choruses recorded for (a) total time spent calling during the 10 min recordings ( $N = 18$ ), (b) total proportion of nonoverlapped calls ( $N = 18$ ), and (c) body condition ( $N = 12$ ). Differences were tested with Wilcoxon tests for paired data. \* $P < 0.05$ ; \*\* $P < 0.01$ .

### EXPERIMENT 3: CALL LEADERSHIP AND BODY CONDITION

#### Methods

In this third experiment we investigated whether leading males, with their more costly form of courtship, had a better body condition, taken as an indicator of available energetic reserves, than following males. To do this, we weighed all 107 males the day after they were captured ( $\pm 0.1$  g). The snout–vent length was measured ( $\pm 0.1$  mm) for each male once the recordings were completed, to avoid stressing the animals through manipulation prior to the recordings. The residuals of the linear regression of the logarithm of weight on the logarithm of snout–vent length were used as indicators of male body condition. However, owing to accidental loss of data, we were able to investigate the relationship between call leadership and body condition for only 12 of the 18 choruses.

To do this, we followed the statistical procedure described for experiment 2, and compared the value of the body condition of the leading male with that of the following male of each chorus using two-tailed exact Wilcoxon tests for paired data. As previously we used R 2.3.0 software and morphological measurements are presented as mean  $\pm$  SD.

#### Results

The 107 captured males weighed  $5.3 \pm 1.5$  g (range 2.6–8.6 g), and snout–vent length was  $42.2 \pm 2.7$  mm (36.5–48.0 mm). Contrary to our expectations, the leading males with their higher energetic investments in calling behaviour did not have a significantly better body condition taken as an indicator of available energetic reserves than the following males (two-tailed Wilcoxon exact test for paired data:  $V = 22$ ,  $N = 12$ ,  $P = 0.204$ ; Fig. 2c).

### DISCUSSION

#### Robustness of Preference for Leading Calls

In our study, female *H. arborea* significantly preferred the first of two identical calls with a 53 ms onset delay. Female preference for the first in a series of overlapping calls has similarly been found in a wide variety of anuran species, including Hylidae (Whitney & Krebs 1975; Klump & Gerhardt 1992), Hyperoliidae (Dyson & Passmore 1988a; Grafe 1996, 1999), Leptodactylidae (Zelick & Narins 1983; Greenfield & Rand 2000), Bufonidae (Howard & Palmer 1995) and Discoglossidae (Bosch & Márquez 2000; Bosch & Márquez 2001).

The strength of this preference is related to the onset delay between competing calls, with females gradually released from auditory masking as this delay increases. In the treefrog *Hyla cinerea*, for example, females chose the first of a series of identical calls broadcast with delays of up to 120 ms (Klump & Gerhardt 1992). In *Hyperolius marmoratus* the critical delay was 70 ms (Grafe 1996). Female preference for leading calls is also relatively independent

of the competing calls' individual characteristics such as amplitude, frequency and duration (Greenfield & Roizen 1993). Thus, in studies conducted on *H. marmoratus* (Grafe 1996; Dyson & Passmore 1988a, b), when presented with a broadcast pattern consisting of two synchronized calls in which the intensity of the leading stimulus had been lowered by 6 dB, females maintained their preference for leading calls when the synchronized calls presented a high degree of overlap. The same was observed when the fundamental frequency of the leading call was elevated by 400 Hz, a modification that should have rendered it a priori less attractive to females. These observations are consistent with studies of acoustic masking in other chorusing anurans and insects (e.g. Greenfield & Roizen 1993; Berg & Greenfield 2005). Tests remain to be conducted on *H. arborea* to determine the effect of the relative intensity, frequency and degree of overlap of competing calls on the strength of female preference for leading signals. However, the consistency of the results obtained to date across a wide variety of species, including Hylidae, strongly suggests that in *H. arborea* as well, males calling too soon after their neighbours suffer a strong penalty as they will be ignored in favour of their leading competitors.

### Costliness of Call Leadership

Our aim in this study was to determine whether male call leadership is a reliable, that is, a costly indicator of quality. The energetic cost of producing leading calls depends on the nature of the physiological mechanism through which males regulate their call timing, the existence of which was first demonstrated in two species of rhythmically calling neotropical treefrogs by Zelik & Narins (1983). The functioning of this mechanism was then elaborated by Greenfield & Roizen (1993) in their study of call timing in the orthopteran *Neoconocephalus spiza*. They showed that, through a central nervous oscillator, males actively regulate their call timing in response to that of their neighbours to maximize the proportion of leading calls they produce and relegate their neighbours' competing calls to an unattractive trailing position. The validity of this mechanism, known as 'inhibitory resetting' has since been extended to many chorusing insects and anurans in which females prefer leading calls (reviewed in Greenfield 1994). However, an important implication of this mechanism relative to the cost of consistently producing leading calls has received surprisingly little attention. In their review of Greenfield & Roizen's inhibitory resetting mechanism, Sheridan et al. (1994) emphasized that (1) male competition to produce attractive leading calls should favour the males with the highest calling rates, and (2) because only the best males will be able to attain and sustain high calling rates, their opponents should be repeatedly inhibited from calling, resulting in leading males producing significantly more nonoverlapped calls (also reviewed in Greenfield 2005). The present study provides novel evidence supporting this predicted. As predicted, the *H. arborea* males with the highest proportion of leading calls in small artificial choruses had significantly higher total calling times which

reflect higher overall call rates than the males with the lowest proportion of leading calls. Moreover, leading males produced a significantly higher proportion of non-overlapped calls.

What implications does this entail for the cost of producing leading calls? As is typical of sexually selected traits, calling is known to be one of the most energetically costly activities in ectothermic vertebrates (Bucher et al. 1982; Taigen & Wells 1985; Ryan 1988; Grafe et al. 1992; Prestwich 1994; Grafe 1997). Among these species, *H. arborea* is exceptional in showing the highest factorial aerobic scope measured so far, with calling metabolism on average 18 times the resting metabolism (Grafe & Thein 2001). In addition, Grafe & Thein (2001) showed that oxygen consumption in this species increases linearly with call rate. Thus, in preferring leading males with higher call rates, females are in effect selecting males with the most energetic courtship song. But is male leadership constrained by male phenotypic quality as measured through body condition?

### Call Leadership and Body Condition

Studies on calling in anurans have shown that male vocalizations are produced by contraction of the aerobic trunk muscles fuelled principally by carbohydrates and lipids stored within these muscles. Species and individuals that call at higher rates exercise their muscles at higher rates and consume greater amounts of fuel reserves in the muscles (Bevier 1997). Thus we predicted that male production of costly leading calls associated with high call rates would be constrained by the quantities of glycogen and lipid reserves in the trunk muscles. Surprisingly, we found no significant relation between the costliness of male calling behaviour (proportion of leading calls) and residual mass taken as an indicator of the quantity of energetic reserves carried by the animal. Ours is not the first study to be confronted with this problem: similar results have been found in several studies that investigated the link between calling performance and body condition in anurans (e.g. Green 1990; M. Reichert, unpublished data). Contrary to what we predicted, this clearly suggests that the production of costly leading calls does not depend on male body condition.

This may be explained by the fact that, in anurans, residual mass may not be the best indicator of male phenotypic quality. Anurans, unlike mammals, birds and fish, carry only a negligible mass of energetic reserves, which represent less than 1% of the animal's total weight (Y. Voituron, personal communication). This restriction of the quantity of carbohydrate and fatty reserves carried is expected to be extreme in arboreal species such as Hylidae. One can also hypothesize that in hylids, the association of the physiological constraints linked to their arboreal lifestyle, their often prolonged breeding season and their extremely costly courtship (Grafe & Thein 2001 measured a depletion of over 63% of carbohydrate reserves in *H. arborea* males after 3 h of calling activity) is likely to result in these species being income breeders, with calling males renewing their metabolic reserves frequently, possibly daily,

during the breeding season. If such were the case, male ability to produce attractive leading sexual displays would probably rest upon individual foraging efficiency, rather than on long-term energetic reserves, which would then be a major determinant of energetic reserves available for sexual advertisement signals and hence the quality of the courtship song produced. In support of this hypothesis, Murphy (1994) found that supplementary feeding of *Hyla gratiosa* males leaving the chorus resulted in these males returning to the chorus faster and remaining there longer than nonfed control males. Further investigation of this question in *H. arborea* is planned.

In addition, the ability to produce expensive calls is not only a function of energetic reserves; individual differences also exist. Grafe & Thein (2001), who measured oxygen consumption during calling in 18 *H. arborea* males, measured up to three-fold differences in oxygen consumption for identical call rates. Thus, the production of calls of varying quality (typically in terms of the proportion of leading calls and call rate) with equivalent energetic reserves is likely to be possible through physiological or morphological individual differences, such as mitochondrial densities in the trunk muscles. This is suggested by studies on moths in which the density of mitochondria present in muscles was related to individual physical condition, and thus physical condition indirectly influenced the efficiency of the individual's aerobic metabolism (Kukal et al. 1989). Physiological studies are now needed to determine whether individual differences in mitochondrial densities in the trunk muscles (involved in sound production in anurans) could partly explain individual differences observed in maximum call rates in *H. arborea* (Grafe & Thein 2001; this study) and many other anuran species. In addition, novel work conducted by Pauly et al. (2006) on túngara frogs, *Physalleanus pustulosus*, has shown that male call rate is also influenced by another organ involved in sound production, the vocal sac. Its role is to return to the lungs the air that they expelled into it during call production. Pauly et al. (2006) suggested that the use of a vocal sac for lung inflation, by increasing the volume of air in the lungs, influences call properties such as the rate of production. This raises the question of whether individual differences in maximal call rates and call leadership might not also be explained by individual differences in the size of the vocal sac.

Thus, our study's focus on male quality in what is probably an income breeding species suggests that factors other than simply body condition determine individual ability to produce energetically costly sexual signals. This stresses the importance of investigating the potential role of various physiological characteristics ranging from foraging efficiency to metabolic efficiency in the determination of male quality in intersexual selection.

### Consequence of Preference for Leading Calls in Natural Conditions

Lastly, the female preference for costly quality-dependent calls shown in this study should lead to adaptive mate choice only when females are able to select these

males in a natural chorus setting. This presents several differences from the laboratory conditions considered in this study. For one, in natural conditions closely synchronized calls will differ in amplitude, and in spectral and temporal structure. Therefore, as mentioned previously, the consistent finding that, in many species, female preference for leading calls is relatively independent of the competing calls' own characteristics is important. In addition, as discussed above, in many species female preference for leading calls depends on the onset delay of the competing calls, with the critical delay, beyond which the preference for leading calls breaks down, varying with the species considered. In this study, on the basis of the results of our phonotaxis test, we estimated male call leadership by considering that a leading call would be preferred on the condition that it overlapped a competing call. However, since in natural interactions the delay between competing calls is variable, to quantify male call leadership exactly, a detailed study of the critical onset call delay under which female preference for leading calls is maintained would be necessary. Besides this is the fact that most studies of the effect of call timing on female preference, including this one, have focused on the experimentally ideal situation of female choice between an isolated pair of equidistant calling individuals. In natural choruses, females are surrounded by many males, calling from various distances and heights. Thus the leading male's relative attractiveness would depend on the relative signal amplitudes of calling males and their relative distances from the female. In one of the few studies conducted on the strength of female preference for leading calls in more realistic conditions, Snedden & Greenfield (1998) showed that in *N. spiza* this preference remained, under certain circumstances, when females were presented with a sequence of four overlapping calls. Their results suggest that female preference for leading calls is sufficiently robust to maintain control over mate choice in a complex acoustic environment.

Very little is known of the functioning of sexual selection in natural choruses of *H. arborea*. Attempts have been made in several chorusing anurans to correlate male mating success (number of matings) over a breeding season with male calling performance (Gerhardt et al. 1987; Bertram et al. 1996; Murphy 2003). Such studies are, however, extremely complex to carry out rigorously since female choice is most probably a function of the quality of a male's calling performance relative to that of the other males present, detected and taken into account by the female at the breeding site, at the time of her choice. Therefore, unless one has recordings of all the males effectively prospected by the female, it is impossible to identify conclusively the call parameters effectively used by the females to choose their mate. Although studies have concentrated on intersexual selection in natural populations of *H. arborea* (Márquez & Tejedo 1990; Friedl & Klump 2005), to our knowledge none of them have taken these factors into consideration. At present we consider that experimental female phonotaxis tests conducted in complex acoustic conditions remain a simpler and more powerful means of studying mate choice in a chorus situation. Experiments to be conducted on



*H. arborea* are therefore under preparation to investigate the conditions under which female preference for leading calls is maintained during mate choice within the complex acoustic environment of an anuran chorus.

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