Hearing is not necessarily believing in nocturnal anurans

Christina Richardson^{1,*}, Doris Gomez²,
Romain Durieux², Marc Théry², Pierre Joly¹,
Jean-Paul Léna¹, Sandrine Plénet¹
and Thierry Lengagne¹

¹UMR-CNRS 5023, Laboratoire d'Ecologie des Hydrosystèmes Fluviaux, Université Claude Bernard Lyon 1, Université de Lyon, Bât. Darwin C, 43 boulevard du 11 novembre 1918, 69622 Villeurbanne Cedex, France

²UMR-CNRS 7179, Muséum National d'Histoire Naturelle, Département d'Ecologie et de Gestion de la Biodiversité, 1 avenue du petit château, 91800 Brunoy, France

*Author for correspondence (nina.richardson@gmail.com).

The recent discovery of the use of visual cues for mate choice by nocturnal acoustic species raises the important, and to date unaddressed, question of how these signals affect the outcome of mate choice predicted by female preference for male calls. In order to address this question, we presented female *Hyla arborea* tree frogs with a series of choices between combinations of acoustic and visual cues of varying quality in nocturnal conditions. While females exhibited the expected preference for a combination of attractive values for visual and acoustic signals over combinations of unattractive values for both signals, when presented with conflicting acoustic and visual cues, they equally adopted one of two strategies, preferring either attractive calls or intense vocal sac coloration. This constitutes novel evidence that the outcome of mate choice, as predicted on the basis of male calling quality, can be drastically different when additional communication modalities—in this case vision—are taken into account. These results also highlight the possible existence of individual variation in female rules for cue prioritization. The implications of these results for the study of mate choice in nocturnal acoustic species are discussed.

Keywords: sexual selection; mate choice; multimodal communication; night vision; anurans

1. INTRODUCTION

Sexual selection through mate choice is a crucial behavioural mechanism with far-reaching evolutionary consequences, since, to a large extent, it determines individual fitness. One field of research that has proven to be extremely rewarding is that of the highly conspicuous sexual calls of nocturnal anurans (reviewed in Gerhardt & Huber 2002). While the study of female assessment of these signals constitutes an ongoing challenge, Rosenthal *et al.* (2004) uncovered a whole new range of sexual signals to be explored, when they showed that female mate choice

is affected by the presence of the inflated male vocal sac. Since then, a handful of studies have concerned themselves with the use of visual cues in various nocturnal anurans and have found evidence of female attention to such cues (Taylor *et al.* 2007; Gomez *et al.* 2009). These exciting preliminary results suggest that the use of visual cues in nocturnal anurans is probably far more widespread than we have so far believed.

However, this also raises an important, and to date unaddressed, question: how do these visual sexual signals affect the outcome of mate choice predicted by female preference for male acoustic signals? If visual signals advertise male traits that present no correlation with those advertised by call parameters, they could interfere with, or even reverse, female assessment of male quality based on acoustic parameters alone. It therefore appears crucial to investigate the rules through which females prioritize multimodal cues in order to reach a realistic understanding of the functioning of mate choice in such species.

The tree frog, *Hyla arborea*, in which sexual selection has been observed to take place through female mate choice (Richardson & Lengagne 2010), constitutes an ideal candidate to address this question. Studies we conducted in this species have shown that, in addition to several acoustic parameters (Richardson & Lengagne 2010), females exhibit a preference for males with a highly colourful vocal sac (Gomez *et al.* 2009). This visual cue was found to be carotenoid-based (Richardson *et al.* 2009), signifying that it is probably a reliable indicator of male immune condition (e.g. Faivre *et al.* 2003). Further, a study of the distribution of call quality and vocal sac coloration we conducted in the large study population (D. Gomez, C. Richardson, M. Théry, T. Lengagne, J.-P. Léna, S. Plénet & P. Joly 2007, unpublished data) found no correlation between the acoustic and visual cues involved in mate choice.

The aim of this study was therefore to examine for the first time female rules of prioritization of multimodal cues. In order to do this, we conducted two indoor two-stimuli experiments in which we presented females with a choice either (i) between a speaker broadcasting attractive calls associated to a video of a male with attractive bright vocal sac coloration, and a speaker broadcasting unattractive calls associated with a video of a male with unattractive pale vocal sac coloration (experiment 1), or (ii) between a speaker broadcasting attractive calls associated with a video of a male with unattractive vocal sac coloration, and a speaker broadcasting unattractive calls associated with a video of a male with an attractive vocal sac coloration (experiment 2).

2. MATERIAL AND METHODS

We investigated female integration of visual and acoustic stimuli in a large metapopulation of *H. arborea* from the Ile Cremieu area, in Southeast France (45°45'10" N, 5°19'56" E). The experiments are described briefly below. For details concerning arena design and electronic equipment, the preparation of the stimuli and female utilization refer to the electronic supplementary material.

(a) Audiovisual signal design

In order to conduct the two experiments, we constructed two synthetic call bouts with attractive and unattractive values, respectively, for call amplitude, rate of emission and peak frequency, using the parameter values for the first and ninth decile of the distributions previously established for the study population (Richardson

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Figure 1. Female preference for attractive (black bars) versus unattractive (grey bars) male calls when (a) attractive calls were associated with attractive vocal sac coloration and unattractive calls were associated with unattractive vocal sac coloration, and (b) attractive calls were associated with unattractive vocal sac coloration and unattractive calls were associated with attractive vocal sac coloration. Differences were tested using two-tailed binomial tests (** $p < 0.01$).

et al. submitted, for details parameter see table S1 in the electronic supplementary material).

We then constructed two 20 min video sequences presenting a male front-on with an inflated non-vibrating vocal sac. Male body coloration was set to the average value found in the local population, whereas vocal sac coloration had a reflectance spectrum that was either that of the first (unattractive pale vocal sac) or the fourth (attractive colourful vocal sac) quartile of the population distribution. For each video the colours were adjusted to match a frog's colour perception following a technique described by Gomez *et al.* (2009).

(b) Experimental procedure

Females were tested at night. At the beginning of each trial a female was placed for 2 min in a visually and acoustically transparent acclimation cage located in the centre of the arena. The female was then released remotely and her movements monitored in infrared light. A choice was scored when the female positioned herself within 10 cm of a screen for 20 s. If no choice was made within 20 min, if the female exited the testing area or if it had not clearly oriented itself towards both of the screens during the test, the trial was discarded and the female was retested later in the night in order to obtain if possible one valid response for each of the two experiments. Between each trial, we alternated the screen and speaker on which the stimuli were presented to control for any possible side biases. After testing, all the females were released on the following day at their site of capture. Female preference was investigated using two-tailed binomial tests with R v. 2.7.0 statistical analysis software (R Development Core Team 2008). Results are presented in the form mean $\pm$ s.d.

3. RESULTS

As expected, in experiment 1 females exhibited a strong preference for an 'all attractive' male over an 'all unattractive' one, with only two out of the 18 females tested choosing the latter ($n = 18$, two-tailed binomial test: $p = 0.0013$, see figure 1a). However, surprisingly, when presented with acoustic and visual cues advertising diverging levels of quality in experiment 2, half the females (10 out of 20) tested preferred high-quality calls associated with a pale vocal sac over low-quality calls associated with a highly colourful sac, whereas the other half chose the opposite ($n = 20$, see figure 1b). Time needed to reach a decision did not differ significantly between the two experiments (260.4 ± 176.3 s in experiment 1 versus 218.3 ± 168.1 s in experiment 2, Wilcoxon test: $W = 119.5$, $p = 0.5411$).

4. DISCUSSION

As expected, when given the choice between a combination of attractive audiovisual cues versus unattractive audiovisual cues (experiment 1), a significant majority of females preferred the 'males' with attractive calls. In contrast with this result, however, when attractive acoustic cues were associated with unattractive vocal sac coloration, and opposed to unattractive calls combined with attractive coloration, only half the females tested exhibited a preference for the males with attractive calls, the other half preferring the more brightly coloured males, despite their unattractive calls.

This study thus challenges the long accepted assumption that communication in nocturnal anurans is mainly acoustic. It clearly demonstrates that the outcome of female mate choice, as predicted on the basis of male calling quality, can be drastically different when additional communication modalities—in this case vision—are taken into account. This result could also help to explain the frequent failure of field studies to find a correlation between male calling behaviour and observed mating success (reviewed by Sullivan & Kwiatkowski 2007). It now appears clear, that in such studies, once issues concerning the rigorous assessment of male acoustic signals have been addressed, a whole new modality of signals needs to be taken into account in order to accurately predict male quality.

In experiment 2, females were confronted with conflicting acoustic and visual cues. Since previous work (D. Gomez, C. Richardson, M. Théry, T. Lengagne, J.-P. Léna, S. Plénet & P. Joly 2007, unpublished data) has found acoustic and visual cues to be uncorrelated for this species in the study population, such a choice is likely one female is frequently faced with in nature. In this experiment, females equally adopted one of two strategies, preferring either attractive calls or intense vocal sac coloration. One interpretation of this result could be, according to the 'sensory overload' theory of Hebets & Papaj (2005), that by providing conflicting acoustic and visual information males are 'jamming' the females' reception and/or processing system, effectively leading them to choose their mate randomly. A second possibility is that females process individual cues to assess overall male quality (Candolin 2003) and that in the present experiment both males, although they exhibited individual cues of differing attractiveness, were evaluated by females as being of equivalent overall quality, leading to females not preferring one male over the other.

Another explanation is that females may differ in the attention they pay to different male qualities and therefore present individual variation in preference rules for the various cues of multiple signals. Data relating to rules through which females prioritize several cues are rare. In her review of the role of multi-component sexual signals, Candolin (2003) suggested that such signals containing 'multiple message' cues could enable females to make a more precise choice of mate, prioritizing cues according to their relative importance. In accord with this theory, in their study of *Gryllus campestris*, Scheuber *et al.* (2004) found that at the population level females preferred calls with attractive spectral characteristics, indicators of high-quality long-term development, over calls with

attractive temporal components indicating good current condition. A more complex possibility is that females may vary individually in the rules through which they prioritize multiple cues, rules that could depend, for example, on female genetic make-up, life-history stage or the cost of mate choice (Candolin 2003). To our knowledge, this is the first study that demonstrates that females may present individual variation in their rules of preference for multiple—and in this case multimodal—cues, with some females favouring attractive acoustic cues, potential indicators of energetic reserves (Prestwich 1994), whereas others prefer bright carotenoid-based vocal sac coloration, a likely indicator of immune condition (e.g. Faivre *et al.* 2003). In addition to the challenge of identifying the various—potentially multimodal—cues involved in sexual communication in nocturnal species, it therefore now appears crucial for our understanding of mate choice that students of sexual selection also take into consideration the possibility of individual variation in female rules of prioritization of these cues using repeatability tests.

This study was conducted in accordance with the current laws in France and with the approval of the Préfecture de l'Isère (decision 2007-03328) and the Direction des Services Vétérinaires (DSV n°69266347).

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