The role of nocturnal vision in mate choice: females prefer conspicuous males in the European tree frog (Hyla arborea)

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doi: 10.1098/rspb.2009.0168

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Nocturnal frog species rely extensively on vocalization for reproduction. But recent studies provide evidence for an important, though long overlooked, role of visual communication. In many species, calling males exhibit a conspicuous pulsing vocal sac, a signal bearing visually important dynamic components. Here, we investigate female preference for male vocal sac coloration—a question hitherto unexplored—and male colour pattern in the European tree frog (Hyla arborea). Under nocturnal conditions, we conducted two-choice experiments involving video playbacks of calling males with identical calls and showing various naturally encountered colour signals, differing in their chromatic and brightness components. We adjusted video colours to match the frogs’ visual perception, a crucial aspect not considered in previous experiments. Females prefer males with a colourful sac and a pronounced flank stripe. Both signals probably enhance male conspicuousness and facilitate detection and localization by females. This study provides the first experimental evidence of a preference for specific vocal sac spectral properties in a nocturnal anuran species. Vocal sac coloration is based on carotenoids and may convey information about male quality worthwhile for females to assess. The informative content of the flank stripe remains to be demonstrated.

Keywords: mate choice; coloration; nocturnal vision; multimodal signalling; vocal sac; lateral stripe

1. INTRODUCTION

Acoustic signals are crucial for animal communication. They are particularly important for reproduction in nocturnal frog and toad species. Males usually gather in choruses (Gerhardt 1994) and produce advertisement calls that are highly variable among individuals (Friedl & Klump 2002). Individuals are able to discriminate fine variations in calling properties (call frequency: Gerhardt et al. 2007; call rate: Marquez et al. 2008; call overlapping: Richardson et al. 2008) and use these cues to adjust their behaviour accordingly. However, additional modalities such as vision are likely to play an important role in communication, as suggested by recent physiological and behavioural studies. Anuran species are endowed with a visual system that is highly sensitive to very low light levels (Aho et al. 1988; Cummings et al. 2008) and is used for prey detection (e.g. Aho et al. 1993; Buchanan 1998). This system is based on two types of rods (review in King et al. 1993), providing them with a colour or light-intensity vision, depending on visual information processing (Kelber & Roth 2006). In addition, many nocturnal anuran species are sexually dimorphic in coloration (Buchanan 1994; Hoffman & Blouin 2000; Sheldon et al. 2003; Vasquez & Pfennig 2007) and present a large repertoire of visual displays (e.g. Amézquita & Hödl 2004)—vocal sac inflation, foot flagging, arm waving—involved in mate attraction and choice (Sheldon et al. 2003; Rosenthal et al. 2004; Taylor et al. 2007; Vasquez & Pfennig 2007) or agonistic interactions (Amézquita & Hödl 2004; Hartmann et al. 2005).

To date, very few studies have explored the influence of male colour intensity or pattern on female mate choice (Sheldon et al. 2003; Rosenthal et al. 2004; Taylor et al. 2007; Vasquez & Pfennig 2007), calling for additional experimental investigation. Studies conducted so far show female preferences for particular colour patterns: a pronounced dark dorsal mottling in the spadefoot toad Scaphiopus couchii (Vasquez & Pfennig 2007); a large lateral stripe in the squirrel tree frog Hyla squirella (Taylor et al. 2007). The vocal sac plays a role in communication, probably because of its pulsing aspect (Rosenthal et al. 2004; Taylor et al. 2007; Cummings et al. 2008). The visual stimulus of a pulsing sac adds to the attractiveness of a non-visible calling male in the squirrel tree frog (Taylor et al. 2007). Females prefer visual displays coinciding with male calling activity, such as males showing a pulsing sac rather than a deflated sac while singing (Rosenthal et al. 2004; Taylor et al. 2007). Although vocal sac coloration has been suggested to enhance male conspicuousness (Cummings et al. 2008), its role in reproductive communication has hitherto never been investigated.

In this study, we experimentally explore female preference for male coloration (vocal sac and colour
pattern) in the European tree frog, *Hyla arborea*, a nocturnal chorusing species in which males display a vocal sac and a dark flank stripe, both variables in coloration among males. Vocal sac pigments are currently being identified in biochemical analyses (Richardson et al., submitted); preliminary results demonstrate that vocal sac coloration is based on carotenoids, as suggested by previous studies (Czeczuga 1980). Because carotenoids are costly pigments involved in defence against pathogens (Olson & Owens 1998), vocal sac coloration would convey reliable information about male quality and be a valuable cue for females to assess. We examine female preference for male colour attributes in two-choice experiments using video playbacks of calling males showing coloration differing in both chromatic and brightness components. We test different naturally encountered signals and adjust stimuli colours in order to match the frogs’ perception of natural colours, a crucial aspect that has never been considered in previous experiments based on male models (Sheldon et al. 2003; Taylor et al. 2007; Vasquez & Pfennig 2007) or in video playbacks (Rosenthal et al. 2004). More specifically, we test the relative attractiveness of a colourful versus a pale vocal sac and the influence of the flank-stripe size on female mate choice. A colourful sac or a large dark flank stripe are more conspicuous signals, and as such can be potentially preferred by females (Bradbury & Vehrencamp 1998).

2. MATERIAL AND METHODS

(a) Female capture

We collected female tree frogs from a metapopulation from the Ile Crémieu area (centre-east France; 5°21’07”E, 45°44’17”N). Females were caught on drift fences or in the water using white headlights; they were collected during nightly choruses and housed in 10.5 l aquaria until they were tested, either the same night or the following night. Females were kept at a natural light cycle in aquaria simulating natural conditions for adults, with pond water and branches with foliage for hydration and resting. Female receptivity was tested at the field site, in an enclosure with a loudspeaker at one end playing a chorus playback. Females showing phonotaxis towards the loudspeaker were considered receptive and selected to perform the tests the same night. The others were either kept to be tested again the following night or released. Receptive females were tested in different experiments, two of which are presented here.

(b) Arena design

Experiments took place in an indoor enclosure to control for ambient noise, light and weather conditions. The arena, designed to test animals in two-choice experiments, consisted of two 20" LCD computer screens (Samsung SyncMaster 2032 BW) forming a triangle with the frog such that the angle of the screens relative to the point where the animal was placed for habituation was 60°. Screens had a large vision angle (170°) to allow animals to view stimuli from a large range of positions. They were covered with black matt foam board except for a 5 × 3 cm rectangle where the visual stimulus was displayed. In front of each screen we placed a loudspeaker (Monacor SP7 connected to a computer via a Stageline 102 amplifier) below the enclosure floor and covered it with 10 × 10 cm thin wire netting, defining a ‘choice area’ where frogs could sit on the loudspeaker and simultaneously watch and eventually touch the screen. We covered the arena with foam pieces to improve sound quality; the foam was covered with wire netting to prevent the frogs from leaving odorant trails. The enclosure’s wooden floor was covered with a cork layer to improve sound quality, and with a removable gunny rinsed in pond water to provide the frogs with a more familiar odorant environment; the gunny was rinsed between any two trials to prevent the frogs from following left scents. The frogs’ movements were recorded in infrared light (Sony HDR-SR7E), to which frogs are blind (Jaeger & Hailman 1973). The video recordings were later analysed.

(c) Video experiments

We built two experiments with videos corrected for sound and colour (explained below). Each experiment involved two males calling antiphonally and showing body movements corresponding to calling activity. The video soundtrack was modified to build synthetic calls identical for both males, with call property values commonly encountered in the local population. Colours were adjusted to match a frog’s colour perception. Male body coloration was set to the average value found in the local population (figure 1), except for the vocal sac coloration that varied according to the experiment.

— Experiment 1 (exp. 1) tested the influence of vocal sac coloration on female choice. It opposed a male with a pale vocal sac to a male with a colourful vocal sac (figure 2a, b). Both were seen in front view and showed an average back and belly coloration.

— Experiment 2 (exp. 2) tested the role of the flank-stripe size on female choice. It opposed a male with a pronounced dark stripe to a male with a reduced stripe (figure 2c, d). Both males were shown in profile and showed an average vocal sac, back and belly coloration.

We expected females to prefer the colourful male over the pale male in experiment 1, and the male with the pronounced flank stripe over the male with the reduced flank stripe in experiment 2. We alternated the screen on which the expected preferred stimulus was presented between any two consecutive trials. All females were tested using a single video for a given experiment, which may create a pseudoreplication problem that may compromise the external validity of the potential results (Kroodsma 1989). Yet the use of synthetic stimuli and the fine control of both the acoustic and visual characteristics of the stimuli provided allowed us to avoid this problem, which typically arises because of a lack of control over the differences in the stimuli tested (McGregor 1992).

(d) Trial protocol

Trials were performed in total darkness between 21.00 and 03.00 hours. Each female performed two trials. These two trials were always two different experiments, randomly assigned to any one female. No female was ever tested twice in the same experiment. The design was thus balanced across all experiments and not only for those presented here; we tested approximately 43 females per experiment but not all females were tested in both exp. 1 and 2.

Throughout the testing process, frog handling involved the use of a dim red headlight, just powerful enough for us to see. Since rods of frogs show an almost null sensitivity in this range of wavelengths (600–700 nm; figure 1), this ensured a minimal disturbance of dark adaptation. Female frogs were
first placed in a habituation chamber (a light-safe, acoustic chamber with a chorus playback of 60 dB sound pressure level (SPL) at 1 m measured using a SL 4001 Lutron sound level meter) for 1 hour before testing, ensuring sufficient acoustic and visual adaptation for the purpose of this study. We then randomly selected one individual and placed it for 2 min in a restraining wire cage equidistant (1 m; sound at 80 dB SPL at 1 m) from both screens. After this period, we removed the cage lid, allowing the female to move freely for 20 min. We considered that the female had made its choice when it remained more than 30 s on the choice area in front of a screen. The choice was not valid if the individual had not sampled both visual stimuli available, stood motionless for 5 min after habituation, left the arena or remained less than 30 s on the choice areas. After their first trial, females were placed back in the habituation chamber for at least half an hour before their second trial. After their second trial, they were measured for snout-vent length ($\pm 0.5$ mm) with a ruler and for body weight ($\pm 0.001$ g) using a Mettler-Toledo PB153 balance before being released where captured. From the 70 receptive females tested in experiments, 60 had their choice validated in at least one trial. Results are presented for all the females, the choice of which was validated in at least one of the two experiments presented here.

**Acoustic stimuli preparation**

We captured several males from the Île Crémieu metapopulation, and video and audio recorded them while calling, one in front view, one in profile. Using AVISOFT, we modified male songs (call duration: 96 ms; dominant frequency: 2629 Hz, fundamental frequency 1052 Hz, 28/34 calls per bout; 5.51/6.31 calls s$^{-1}$ for the front/profile view). We also constructed two 15 s video basic sequences, one (6 s song, 1.5 s silence, 6 s silence, 1.5 s silence) on one screen, the other (6 s silence, 1.5 s silence, 6 s song, 1.5 s silence) on the other screen. In song periods, male calls were synchronized to

![Figure 1](http://rspb.royalsocietypublishing.org/content/276/1695/2353.full.pdf)

**Figure 1.** Reflectance spectra for the mean vocal sac (bold plain curve with standard deviations), the back (short-dashed curve), the belly (long-dashed curve) and the flank-stripe (i.e. the reflectance of the dark area underlining the light line composing the stripe; thin plain curve) coloration of male European tree frogs from the Île Crémieu metapopulation. The flank stripe was measured on $n=2$ males, while all other body areas were measured on $n=125$ males. Relative absorption functions of rods sensitive to short (plain grey curve) and middle (dashed grey curve) wavelengths used to model the European tree frog vision (see §2 for details).

![Figure 2](http://rspb.royalsocietypublishing.org/content/276/1695/2353.full.pdf)

**Figure 2.** Visual stimuli displayed in mate choice experiments. Exp. 1 opposed (a) a male with a pale vocal sac to (b) a male with a colourful vocal sac, both of average dorsal coloration. Exp. 2 opposed (c) a male with a pronounced flank stripe to (d) a male with a reduced flank stripe, both of average coloration. The configuration shown here presents (a,c) stimuli on the left screen and (b,d) stimuli on the right screen. The reverse configuration was also used and the configuration was alternated between any two consecutive tests. Males differed in their red appearance from one experiment to another, but this was assumed to have a negligible influence on the perceived colour (see §2 for details).

**Acoustic stimuli preparation**

We captured several males from the Île Crémieu metapopulation, and video and audio recorded them while calling, one in front view, one in profile. Using AVISOFT, we modified male songs (call duration: 96 ms; dominant frequency: 2629 Hz, fundamental frequency 1052 Hz, 28/34 calls per bout; 5.51/6.31 calls s$^{-1}$ for the front/profile view). We also constructed two 15 s video basic sequences, one (6 s song, 1.5 s silence, 6 s silence, 1.5 s silence) on one screen, the other (6 s silence, 1.5 s silence, 6 s song, 1.5 s silence) on the other screen. In song periods, male calls were synchronized to
vocal sac pulsations; in silence periods, males stood motionless with their vocal sacs inflated. For a given experiment, acoustic stimulations given on both screens were strictly identical and antiphonal with no overlap, a property known to affect male attractiveness in H. arborea (Richardson et al. 2008). We controlled for sound amplitude before each trial, so that females could only rely on visual but not on acoustic cues to choose their mate.

(f) Video stimuli preparation

Video screens are designed for human vision and fail to render natural colours as perceived by animals with a different visual sensitivity. Modifying video colours to create a perceptual match for the animal vision between ‘natural’ and ‘video’ stimuli is a crucial procedure when using video playbacks (Fleishman et al. 1998). This was a two-step procedure: (i) determination of the colour signals naturally encountered in males in the Île Cré´mieu metapopulation, and (ii) adjustment of the RGB values of each colour patch on the videos to render colours as perceived by frogs at night. We did so using MATLAB and Adobe PREMIERE.

First, we determined which colours to display. A total of 125 males had been captured from the Île Cré´mieu metapopulation in the year before the experiment. Their back, belly and vocal sac coloration had been measured in reflectance spectrometry, using a spectrometer (Avantes AvaSpec-3648-SPU2), a deuterium-halogen light source (Avantes AvaLight-DHS) and a coaxial optic fibre (Avantes FCR-7UV200-2-45-ME). The same equipment was used to measure the flank-stripe coloration in a few males captured for other purposes in the year of the experiment and displaying a stripe large enough to be measured with our equipment. We analysed spectra using AVICOL (Gomez 2006) and extracted spectra mean (m) and standard deviation (σ). We chose m for back and belly coloration, m for an average vocal sac, (m+σ) for a pale vocal sac and (m−σ) for a colourful vocal sac (figure 1), thus creating stimuli that differed by their chromatic and achromatic components. We also analysed spectra by computing brightness (mean reflectance over the range 350–700 nm) and chroma (difference between minimal and maximal reflectance over the mean reflectance over 350–700 nm), two parameters commonly characterizing spectral shape (e.g. Doutrelant et al. 2008). Spectra for pale and colourful vocal sacs had brightnesses of 14.73 and 8.28, and chroma of 0.7 and 1.1, respectively.

Second, we adjusted RGB combination for each colour patch independently to match a frog’s vision. The visual system of the European tree frog is unknown, but two rod classes peaking at 435 and 503 nm have been identified in the Green tree frog Hyla cinerea (King et al. 1993), and are involved in nocturnal vision through two possible mechanisms. An antagonistic processing of rod outputs would give a colour sensitivity, while an additional processing would give a brightness sensitivity. Because we do not know whether frogs perceive variations on achromatic aspects (colour vision) or only on achromatic aspects (brightness), we created stimuli that varied regarding these two aspects: (i) to optimize the probability that the variation would be detected, and (ii) to explore the natural range of variation of visual signals.

Whatever the neural processing performed, a video stimulus and a natural stimulus are perceived as identical if they elicit the same quantity and ratio of neural output from the different classes of photoreceptors in the animal’s eye (Fleishman et al. 1998). We thus had to find the (C_R;C_G,C_B) combination that satisﬁes the equation

\[ \text{with } R_{\text{male}}(\lambda) = \text{the reflectance spectrum of the back, belly or vocal sac of the male; } I_{\text{moonlight}}(\lambda) = \text{the moonlight irradiance spectrum, which we digitized from Warrant (2004) using WinDigt (Lovy 1996); } S_{\text{rod1}}(\lambda) \text{ and } S_{\text{rod2}}(\lambda) = \text{the absorbance spectra of the tree frog rods (figure 1); and } R_{\text{back}}(\lambda), R_{\text{green}}(\lambda) \text{ and } R_{\text{red}}(\lambda) = \text{the radiance spectra of the screen blue, green and red phosphors measured on the screen, with screen brightness set to its minimal value}. \]

We measured absolute radiance spectra of screen phosphors using a spectrometer (Avantes AvaSpec-3648-SPU2) calibrated in mmol m\(^{-2}\) s\(^{-1}\) with a calibration lamp (Avantes AVALIGHT-DH-CAL), an optic fibre (FC-UV600-2-ME) and a cosine-corrected sensor (Ocean Optics CC3-UV). We built rod absorption functions based on Govardovskii’s templates for vertebrate A1 pigments (Govardovskii et al. 2000) and on the lens transmission spectrum established for the Northern leopard frog Rana pipiens, the only anuran species for which these data were available (Kennedy & Milkman 1956).

Since computer screens are designed for human diurnal vision, it was impossible to find an RGB combination that would reproduce moonlight intensity because it was too low. A factor k of 20 000 was needed in order to find a combination (C_B;C_G,C_R) that could be implemented. Such a correction was expected to increase light intensity close to the screen but not much at larger distances, given that light is attenuated with distance (Bradbury & Vehrencamp 1998).

Because equation (2.1) had two equations but three unknown variables, we could find a combination of (C_B;C_G) for any possible value of C_R between 0 and 255. We thus left the value of C_R to its value in the original video. We set (C_B;C_G) to (72,18) for an average vocal sac (m), (90,30) for a pale vocal sac (m+σ), (52,13) for a colourful vocal sac (m−σ), (95,10) for the back and (156,81) for the belly and (56,16) for the flank-stripe coloration. Although similar in average to a dark vocal sac, the flank stripe was highly heterogeneous in coloration; near the eye, the value was approximately (30,10).

In order to assess how realistic the manipulated visual stimuli were in terms of light intensity, we measured the light intensity available at the release point (1 m away from the screen) using two complementary methods. (i) A luminance meter (LMT LI009) gave a direct measurement of the light available. Because the measurement may have included some dark parts of the viewing scene, leading to a possible underestimation of the light provided by the stimuli, (ii) we measured the light close to the screen using the spectrometric device described above. We computed the light intensity at 1 m using Bouguer’s law, according to which the light intensity of a punctual source declines with the inverse of squared distance. Although the source was not a punctual source, this approximation gave a convenient reference for comparison and allowed us to detail the contributions of the entire visual stimuli, the brightest spot of the visual stimuli that determined eye adaptation and parts of the signals tested in the experiments (vocal sac or lateral line). We compared these values to the radiance of the colour patches of an.
average male seen under moonlight. The light provided at 1 m was comparable with the light provided by a male seen in nature, suggesting that the manipulated signals were realistic at that distance in terms of light intensity. The luminance meter gave satisfying estimates of light intensity with no particular underestimation (table 1). Manipulated signals varied not only in chromaticity but also in brightness: the male with the intense vocal sac and the male with the pronounced line were darker than the stimuli they were opposed to (from 30% to 60% decrease in light intensity as expressed in lux in table 1).

(g) Statistical analysis
For each experiment, the null hypothesis posited the absence of female preference for either of the stimuli presented. The observed choices were thus compared with expected equal frequencies in a G-test corrected for small counts. Since variables did not follow a normal distribution, we performed non-parametric tests. All statistics were performed using R v. 2.7.0 (R Development Core Team 2008).

3. RESULTS
Females significantly preferred the male with a colourful sac over the male with the pale sac (exp. 1: n=24 (17/7), G=4.209, p=0.040) and the male with the pronounced flank stripe over the male with the reduced flank stripe (exp. 2: n=25 (18/7), G=4.911, p=0.027). In both experiments, females expressed a preference for the darkest stimulus provided (table 1). A large proportion of females touched the preferred male, as a tactile inspection of the male or amplexus solicitation (71% and 56% for exp. 1 and 2, respectively). Female body mass or size was not related to the day of the test (Spearman p=0.36). Females rapidly made their choice: they took an average of 3 s over the male with the reduced flank stripe.

<table>
<thead>
<tr>
<th>visual stimulus</th>
<th>light intensity 10^{-4} \mu mol m^{-2} s^{-1} (10^{-3} lx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>exp. 1</td>
<td></td>
</tr>
<tr>
<td>male with pale vocal sac</td>
<td>0.82 (1.99)</td>
</tr>
<tr>
<td>male with intense vocal sac</td>
<td>0.80 (3.04)</td>
</tr>
<tr>
<td>exp. 2</td>
<td></td>
</tr>
<tr>
<td>male with pronounced lateral line</td>
<td>0.48 (2.20)</td>
</tr>
<tr>
<td>male with reduced lateral line</td>
<td>0.60 (2.83)</td>
</tr>
<tr>
<td>male under moonlight</td>
<td>0.60 (1.65)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>exp. 1</th>
<th>entire male</th>
<th>brightest spot</th>
<th>vocal sac</th>
</tr>
</thead>
<tbody>
<tr>
<td>male with pale vocal sac</td>
<td>0.08 (0.42)</td>
<td>0.33 (1.96)</td>
<td>0.08 (0.16)</td>
</tr>
<tr>
<td>male with intense vocal sac</td>
<td>0.05 (0.15)</td>
<td>0.24 (1.1)</td>
<td>0.05 (0.044)</td>
</tr>
<tr>
<td>exp. 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male with pronounced lateral line</td>
<td>0.08 (0.58)</td>
<td>0.39 (2.75)</td>
<td>0.14 (0.51)</td>
</tr>
<tr>
<td>male with reduced lateral line</td>
<td>0.11 (0.82)</td>
<td>0.39 (2.8)</td>
<td>0.22 (0.81)</td>
</tr>
<tr>
<td>male under moonlight</td>
<td>0.60 (1.65)</td>
<td>1.02 (2.4)</td>
<td>0.54 (1.2)</td>
</tr>
</tbody>
</table>

4. DISCUSSION
(a) Female preference for male colour characteristics
Given equally attractive calls, females prefer males with a colourful and intense (darker and more chromatic) vocal sac over a pale (lighter and less chromatic) vocal sac. To our knowledge, this is the first experimental evidence of mate choice based on vocal sac coloration in a nocturnal species, when acoustic modalities and body size are controlled for and cannot be used by females to assess their mate quality. In the second experiment, females discriminated between different colour patterns and preferred the male with the more pronounced flank stripe over the male with the reduced flank stripe.

Female preferences for male colour characteristics showed no relation to female body mass or size. This result is in agreement with previous findings on visual sensitivity. Female visual sensitivity varies with female reproductive state in túngara frogs: females ready to lay eggs show similar sensitivity whatever their size is, while non-reproductive females vary in visual sensitivity with their size (Cummings et al. 2008). The absence of variation with female biometry is not surprising, given that in our experiment all tested females were reproductive (that is, searching a mate and ready to lay eggs).

In both experiments, females preferred the darkest stimulus provided, given the choice between two stimuli slightly differing in brightness. This is the expression of a real preference for a particular colour signal and cannot be interpreted as phototaxis (in this case negative phototaxis). Two arguments support this interpretation: (i) the same females expressed preferences for the brightest stimulus in another experiment (results not shown here), suggesting that the direction of phototaxis would be unstable at similar light-intensity levels; and (ii) a natural positive phototaxis is highly frequent in nocturnal anurans and has been observed in Hylids (Hailman & Jaeger 1974), contradicting the preference shown in the experiments of the present study.

Table 1. Light intensity measured from visual stimuli.a

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**Proc. R. Soc. B** (2009)

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As a reference, moonlight intensity is 3.5 x 10^{-3} \mu mol m^{-2} s^{-1} (16 x 10^{-3} lx).

The stimulus was measured close to the screen and the value was then extrapolated at 1 m using Bouguer’s law. Light intensity at 1 cm from the screen can be obtained by multiplying the values presented here by 10 000. The brightest spot was a white spot due to specular reflection.

The data for the entire visual stimulus were measured at 1 m using the luminance meter. This value should be multiplied by 2 to get the overall intensity coming from both screens. All values are over the range 380–700 nm.

As a reference, moonlight intensity is 3.5 x 10^{-3} \mu mol m^{-2} s^{-1} (16 x 10^{-3} lx).

The data for the entire visual stimulus were measured at 1 m using the luminance meter. This value should be multiplied by 2 to get the overall intensity coming from both screens. All values are over the range 380–700 nm.

In both experiments, females were successful in both exp. 1 and 2, we could not test whether they took a similar length of time to choose.
Vocal sac coloration probably conveys reliable information about male quality. Male vocal sac coloration is based on carotenoids (Czeczuga 1988; Richardson et al., submitted), pigments costly to acquire and express (Olson & Owens 1998). Female preference for more intense vocal sac coloration probably reflects a preference for better quality males that can afford to invest more carotenoids in coloration. Carotenoids must be ingested through food intake, which makes them potential indicators of foraging efficiency. Additionally, competition for carotenoids between ornaments and immune function, in which these pigments are involved, may enforce honesty on carotenoid-based signals (Lozano 1994; Von Shantz et al. 1999), as evidenced in the blackbird Turdus merula (Faiivre et al. 2003). Female preference for specific naturally occurring color signals (lateral stripe and vocal sac coloration) suggests that, if male coloration is heritable, female preference may play an important role in shaping male visual signalling displays and behaviour through intersexual selection. As underlined by Friedl (2006), we cannot infer any conclusion about the existence and the direction, if any, of the selection females might exert on male coloration unless we explicitly examine the differences in mating success and survival of males bearing various coloration.

Although not directly tested in our experimental design, the relationship between male colour intensity and male quality or male success has been established in several nocturnal anuran species, underlining the potential for male coloration to be used by females to assess the quality of their potential mate. Experimental studies have shown female preference for redder males (and also for males that are larger and in better condition) in the spadefoot toad S. couchii (Vasquez & Pfennig 2007) and for males with a larger stripe in H. squirella (Taylor et al. 2007). Correlative studies have related coloration to male success: males with a more extended ventral coloration have a higher reproductive success in Cook’s robber frog Eleutherodactylus cooki, a cave-dwelling species (Burrowes 2000). Males with a brighter blue coloration gain a higher reproductive success in moor frogs Rana arvalis (Hedengren 1987) and sire offspring with a better survival, which indicates that coloration may also signal for potential genetic benefits (Sheldon et al. 2003).

(b) Multimodal communication
How females assess multimodal signals and reach their mating decision remains an interesting question to be studied. Acoustic features are prominent in anuran reproductive communication (Ryan 2001), and visual signals may, first, increase male detection and localization and, second, reinforce (redundant signal hypothesis) or complement (multiple messages hypothesis) the information provided by acoustic signals on male quality (Møller & Pomiankowski 1993; Candolín 2003).

Females may encounter difficulties in locating males based only on acoustic cues in a noisy chorus consisting of conspecific and heterospecific calls. Other studies have shown a preference for multimodality (auditory and visual signals) over unimodality (auditory signals only) (Rosenthal et al. 2004; Taylor et al. 2007). Multimodality probably enhances male detectability (by affecting time detection, detection threshold or detection probability) facilitates species recognition and increases memorability of signals by the receivers: different aspects largely documented by experiments on humans and animals (review in Rowe 1999). Improvement of male detectability is achieved not only by the provision of visual signals in addition to acoustic signals, but also by the organization and display of the visual signals themselves. More specifically, a large flank stripe increases the within-pattern visual contrast, at least at short distances (1 m in our experiment), by introducing a dark patch that highly contrasts with the ventral, dorsal and vocal sac patches. Visual contrast enhancement is an efficient strategy to increase conspicuousness for conspecifics (Gomez & Théry 2007). Calling males stand with their vocal sac inflated slightly above the water level and display their lateral parts prominently (Taylor et al. 2007; C. Richardson 2008, personal observation). The lateral stripe is thus particularly visible for females approaching males; it probably increases their detectability in a way similar to how the increase in prey visual contrast reduces detection time in toads (Roberts & Uetz 2008). The lateral stripe may also act as an amplifier (Hasson 1989) and facilitate female assessment of an informative trait such as vocal sac coloration. Two arguments support this view: the lateral stripe constitutes a lateral extension of the vocal sac signal, which can be seen by females from a large range of positions; also, the stripe is concealed by the fore- and hind limbs during daytime, in rest or water conservation posture (Taylor et al. 2007; C. Richardson 2008, personal observation). Restricting the exposure to nightfly courtship displays is a strategy to reduce the higher predation costs associated with conspicuous displays (Bradbury & Vehrencamp 1998).

Apart from improving initial detection, visual signals probably convey information about individual quality, as shown above. This information may either support or complement the information provided by acoustic signals. We cannot provide evidence in support of either the redundant signal hypothesis or the multiple messages hypothesis, given that only one modality varied in our experimental design. It would be interesting to investigate the correlation between visual and acoustic signal expressions in natural populations, as well as mating preferences in more realistic contexts of synchronous variations of different modalities (acoustic and visual). Nevertheless, whatever the interplay between sensory channels, the provision of an increased amount of information probably helps females to choose their mate.

(c) Female behaviour and the use of video playbacks
We investigated female mating preference using video playbacks, a technique increasingly used for behaviour experiments (e.g. in frogs; Rosenthal et al. 2004; review in Trainor & Basolo 2000), with advantages but also limitations (Trainor & Basolo 2000; Robinson-Wolraft 2006).

Videos offer substantial advantages. First, they provide more realistic stimuli than many models used in behaviour experiments (robotic frogs in Narins et al. 2003; frogs with sac inflation controlled by hand in Taylor et al. 2007, 2008; static clay models in Vasquez & Pfennig 2007). They give a better rendering of vocal sac shape, coloration and spatio-temporal properties (pulsations exactly synchronized to sound), which are important parameters, salient to females (Rosenthal et al. 2004), that potentially affect...
male attractiveness. Second, it is easier to dissociate and manipulate independently acoustic and visual modalities and to control for coloration on videos compared with live animals. No study to date conducted on frogs and using models or videos has ever attempted to match live frog coloration (Rosenthal et al. 2004; Taylor et al. 2007; Vasquez & Pfennig 2007). Videos are built for human diurnal trichromatic vision and fail to render colours as perceived by other animals with a different visual system. Adjusting screen colours is crucial to allow for this perceptual mismatch (Fleishman et al. 1998). Our study is the first to adjust video colours for a nocturnal species. We did so by including nearly all the range of frogs’ visual sensitivity, by providing a light-intensity level plausible in natural conditions—at least at the point where females first assessed male coloration—and without making any assumption about how visual information is processed by frogs’ brains.

Several limitations may have affected female behaviour and choice. First, the light source was restricted and not diffuse, which resulted in an artificial increase in intensity at very close distances. Second, the lack of depth cues, which is typical of videos, may have influenced female behaviour by increasing male search, tactile inspection or solicitation, behaviours that we indeed occasionally observed. However, the use of videos can be validated by comparing female (i) behaviour and (ii) mating preferences in the arena and in the wild. Several arguments support our confidence that videos are a good technique for investigating mating preferences in the European tree frog. (i) Females spent a reduced length of time for mate choice, comparable with that found in previous experiments conducted with loudspeaker only (Richardson et al. 2008). (ii) They tend not to pay a close visit to males they do not choose. Similarly, in natural populations, females assess different males for a few minutes (Hyla versicolor, Schwartz et al. 2004; H. arborea, Friedl & Klump 2005) and then move directly towards an individual male without approaching other males (Hyperolius marmoratus, Grafe 1997; H. arborea, Friedl & Klump 2005). (iii) A large proportion of females touched the male they chose. In European tree frogs, amplexus is always initiated by a female nudging a male, and males do not stop calling or try to mount females unless they are touched by females (Friedl & Klump 2005). Video males are thus attractive enough to evoke amplexus solicitation by females. We did not investigate female preferences for visual stimuli in the wild, leaving this question for future research. Yet a preference for a large lateral stripe has been found in natural conditions in H. squirella, a closely related species (Taylor et al. 2007).

This study was conducted with the approval of Préfecture de l’Isère (decision 2007-03328), in accordance with the current laws in France and under a Direction des Services Vétérinaires permit (DSV no. 69266347).

This work was funded by the French national research agency, ANR Colapse. We warmly thank Anais Albert and Aurelien Beigener for their precious help and support, and Antonis Andreou for his technical suggestions. We thank Nadine de Block and Corinne Goninet for their help during the field campaign, and Eric Dumoulin for giving us access to the experimental room, and Rafael Quesada for his extensive knowledge of tree frog biology and populations, as well as the mayors of Optevoz and Anmoisin-Chatelans for giving us access to the field sites. We are grateful to Françoise Vienot for her technical and conceptual contribution in the measurement of light intensity, and to two anonymous reviewers for their comments.

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