Sexual selection in the squirrel treefrog *Hyla squirella*: the role of multimodal cue assessment in female choice

RYAN C. TAYLOR*†, BRYANT W. BUCHANAN‡ & JESSIE L. DOHERTY§

*Department of Biology, University of Louisiana at Lafayette
†Section of Integrative Biology, University of Texas at Austin
‡Biology Department, Utica College, Utica, NY
§U.S. Geological Survey, National Wetlands Research Center, Lafayette, LA

Anuran amphibians have provided an excellent system for the study of animal communication and sexual selection. Studies of female mate choice in anurans, however, have focused almost exclusively on the role of auditory signals. In this study, we examined the effect of both auditory and visual cues on female choice in the squirrel treefrog. Our experiments used a two-choice protocol in which we varied male vocalization properties, visual cues, or both, to assess female preferences for the different cues. Females discriminated against high-frequency calls and expressed a strong preference for calls that contained more energy per unit time (faster call rate). Females expressed a preference for the visual stimulus of a model of a calling male when call properties at the two speakers were held the same. They also showed a significant attraction to a model possessing a relatively large lateral body stripe. These data indicate that visual cues do play a role in mate attraction in this nocturnal frog species. Furthermore, this study adds to a growing body of evidence that suggests that multimodal signals play an important role in sexual selection.

Numerous studies have shown that females across diverse taxa express strong mating preferences for male secondary sexual characteristics (Andersson 1994). Females typically prefer males that possess exaggerated characters such as tail feather length/ornamentation (Andersson 1982; Petrie & Halliday 1994), brightly coloured pigmentation (Hamilton & Zuk 1982; Houde 1987) or larger song repertoire (Hasselquist et al. 1996), and these preferences can provide a selective pressure driving the evolution of male traits.

Preference experiments are often conducted by presenting females with a dichotomous choice where a single property of interest is manipulated (e.g. Basolo 1990; Gerhardt 1991). For example, in many phonotaxis experiments with anuran amphibians, females are presented

with two alternative calls that vary in a single property such as frequency (Gerhardt & Huber 2002). These two-stimulus tests are critical for identifying underlying female preferences. Inferences from these studies on the selective strength that female choice exerts on males, however, may be limited. In nature, females often must assess potential mates in noisy environments, which may limit their ability to detect or discriminate among individual signals (Gerhardt & Klump 1988; Telford et al. 1989; Wollerman 1999; Schwartz et al. 2001; Gerhardt & Huber 2002). Inferences regarding female choice based on the outcome of two-stimulus tests may also be limited because sexual displays often involve different signal components. These multicomponent signals may be transmitted in the same modality such as visual assessment of colour and courtship display in guppies (Kodric-Brown & Nicoletto 2001). Multicomponent signals may also be transmitted in separate modalities such as visual and seismic cues produced by courting jumping spiders (Elias et al. 2005) or visual and olfactory cues in swordtail fish (Crapon de Caprona & Ryan 1990). More recently, work has shown

Correspondence and present address: R. C. Taylor, Department of Biological Sciences, Salisbury University, Salisbury, MD 21801, U.S.A. (email: rctaylor@salisbury.edu). J. L. Doherty is now at the P.O. Box 19, Grantsville, UT 84029, U.S.A.

that females across many taxa assess multicomponent signals (for review see Rowe 1999; Candolin 2003) and a female's mate choice is likely to result from the assessment of multiple signals. Therefore, studies that assess female preferences for a single cue may fail to elucidate patterns of mating because they occur when females have a broader range of information available to them.

Anuran amphibians have provided a model system for the study of acoustic communication and sexual selection (Ryan 2001; Gerhardt & Huber 2002). Relatively little attention has been paid, however, to the role of vision in their breeding behaviours (reviewed in Hödl & Amézquita 2001), particularly in species with nocturnal courtship (Buchanan 1993a). A number of studies have documented visual signalling and suggested that colour changes (Wells 1980) or ritualized movement of body parts (Lindquist & Hetherington 1996; Haddad & Giaretta 1999; Hirschman & Hödl 2006) are likely to be important anuran social signals. Fewer studies, however, have quantified receiver responses to visual signals. Summers et al. (1999) showed that females of *Dendrobates pumilio* express preferences for individuals of their own colour morph. Narins et al. (2003, 2005) showed that males of *Epipedobates femoralis* engage in agonistic encounters only when presented with both auditory and visual signals of a calling male. Rosenthal et al. (2004) showed that females of *Physalaemus pustulosus* showed a preference for male calls that were accompanied by a video playback of a calling male. Amézquita & Hödl (2004) showed that foot-flagging displays in males of *Hyla parviceps* elicited similar displays in an opponent frog.

Despite an increasing interest in the role of visual signalling in anurans, studies have focused largely on diurnal species (but see Amézquita & Hödl 2004; Rosenthal et al. 2004). The lack of attention to visually mediated behaviours for nocturnal species may have stemmed not only from the successes of research on acoustic communication but perhaps also from observer bias (Buchanan 1993a; Elias et al. 2005). Because humans see poorly under nocturnal conditions and because artificial lighting may alter the expression of visually mediated behaviours, researchers may have been less likely to detect visually mediated social behaviour in nocturnal species.

Data on visually mediated behaviour have shown that anurans are capable of seeing objects at illuminations below the level at which humans are able to see. For example, Larsen & Pedersen (1982) showed that the toad *Bufo bufo* is able to strike accurately at prey dummies at illuminations as low as 1×10^{-5} lx, and Buchanan (1998) showed that squirrel treefrogs are able to detect moving prey items at 6×10^{-5} lx using vision alone. Therefore, it does not appear that low light levels would be an impediment to using visual information during courtship at night.

The goals of this study were to (1) test female preferences for properties of male vocalizations in the squirrel treefrog and (2) test the hypothesis that female choice is influenced by visual cues in this nocturnally reproducing species. The squirrel treefrog is a common species occurring throughout the coastal plains of the southeastern United States and typically occupies forest habitats containing a vegetative under-story. Most individuals are active only at illuminations below 10^{-3} lx (Buchanan 1992). Breeding is tied strongly to rain events and occurs only in ephemeral pools during the spring and summer months. Squirrel treefrogs also display sexual dimorphism for labial and lateral stripes with males showing larger and more brightly coloured labial and lateral stripes than females (Buchanan 1994). Thus, this species provides a good model for studies of courtship involving visual cues (Buchanan 1994).

GENERAL METHODS

We collected male and female squirrel treefrogs from two field sites in south-central Louisiana: Thistlethwaite Wildlife Management Area in Saint Landry Parish and Sherburne Wildlife Management Area in Saint Martin Parish. The sites were separated by approximately 40 km. The use of frogs from two areas was necessary to obtain sufficient sample sizes to perform experiments in a year with sporadic reproductive activity. Although not explicitly tested; we noted no differences in call characteristics or behaviour of frogs from these two areas and we do not know whether or not these sites represent distinctly different populations. Individual frogs were collected from vegetation in the forest during daylight hours or in nightly choruses. We housed the frogs in 38-litre aquaria on the campus of the University of Louisiana at Lafayette until they were used in experiments. Our experimental population consisted of approximately 320 individuals (65% from Thistlethwaite and 35% from Sherburne). The frogs were maintained on a 14:10 h light:dark cycle and fed crickets dusted with vitamin powder ad libitum. A plastic bowl of aged tap-water was kept in each aquarium to allow the frogs to hydrate at will. Males were housed at a density of 15−20 per aquarium and females were housed at a density of 12−15 per aquarium (females being slightly larger). In captivity, this species does not show obvious aggression under these conditions and remains stationary most of the time, unless feeding. The frogs showed no obvious signs of stress while housed in the laboratory under these conditions.

We conducted all experiments in an outdoor, wooden-framed enclosure (2.5 × 4 m and 2 m) covered by black aluminium window screening. We covered the outside of the enclosure with black polyethylene plastic to minimize light intrusion from the high-pressure sodium vapour lamps at the research facility. We left the top of the enclosure uncovered (by plastic) to allow natural light to enter from above. The floor of the enclosure was lined with black plastic, creating a 20-cm-deep pond. All experiments were conducted between 2030 and 0500 hours from June−August 2002 and May−August 2003. During late spring and summer, the nightly air temperatures in south Louisiana do not normally fluctuate widely. The mean air temperature for all experiments was $23.8°C \pm 1.17$ SD (range 22.1−26.3°C).

For each night of testing, we placed approximately 70 male and 35 female squirrel treefrogs in the enclosure at 0400 hours and allowed them to acclimate during the day.

Ten large dwarf palmetto, *Sabal minor*, leaves provided refuges for the frogs in the enclosure. Call sites for males consisted of palmetto leaves, concrete cinder blocks, and floating blocks of wood. To stimulate a chorus and to bring females into reproductive condition, we turned on an overhead sprinkler system at 1400 hours each day to create artificial rainfall for approximately 2 h. At dusk (approximately 2030 hours) we began broadcasting a tape of synthetic male vocalizations and allowed the tape to loop for approximately 2 h. This procedure generated a chorus of a stable number of calling males (approximately 35–40) by 2230 hours. Frogs were allowed to chorus until 0030 hours when we entered the enclosure and collected all the frogs. On average, approximately 10–12 females (of the 35 females placed into the enclosure) were found in amplexus with a male and these were the females we tested that night. Preliminary experiments revealed that almost 100% of nonamplectant females failed to respond to playbacks. This failure to respond may be due to lower levels of circulating hormones (Lynch et al. 2006), thus affecting the females' motivational state; therefore, we tested only amplectant females. After collecting the frogs in the enclosure, we placed females from amplectant pairs into opaque plastic cups until testing that night. Because of limitations on the number of females we could care for properly in the laboratory, some females were tested in more than one experiment. No female was ever tested twice in the same experiment. When females were retested, there was a minimum of 14 days between each experiment.

We recorded vocalizations of males from the Thistlethwaite site using a Sennheiser KD5/ME88 shotgun microphone and a Sony WMD-3 recorder. We recorded the calls of 98 males between 2230 and 0300 hours from June to August 1992 during choruses of 10–40 males calling in a 5×5 m screened enclosure (Buchanan 1993b). We determined frequency characteristics and call rates for these calls using a Kay DSP 5500 Sona-Graph. We also recorded the advertisement calls of 38 males in the field at Thistlethwaite Wildlife Management area in 1991. We obtained detailed temporal characteristics of these calls using SoundFX (Silicon Shack) and a Silicon Shack Engineering A:D sound board. Because of availability of analysis software at different times, we were unable to determine all call characteristics for every call. Calls used in playback experiments were synthesized with the assistance of H. C. Gerhardt using custom software created by G. Klump and H. C. Gerhardt and using the program SoundFX (Silicon Shack). Calls were saved to cassette tape using a Silicon Shack Engineering A:D sound board. We played the synthetic vocalizations to females through a Sony WMD-6 cassette recorder, amplified by an RCA model STA 3850 stereo receiver, and broadcast by two RCA model PRO-X33AV loudspeakers. We observed females using an infrared viewer (Night Owl Optics™ infrared viewer, IR) so that visible light would not alter the females' behaviour.

Calls of male squirrel treefrogs possess two frequency peaks, an upper and lower peak (Fig. 1, Table 1). We correlated the average dominant frequencies for the upper and lower dominant frequency peaks with snout–vent length (SVL) and found significant negative relationships (lower: $r = -0.248$, $P = 0.031$, $N = 7$; upper: $r = -0.334$, $P = 0.003$, $N = 76$), demonstrating a relationship between body size and dominant frequency, with larger males having lower-frequency advertisement calls. In our playback
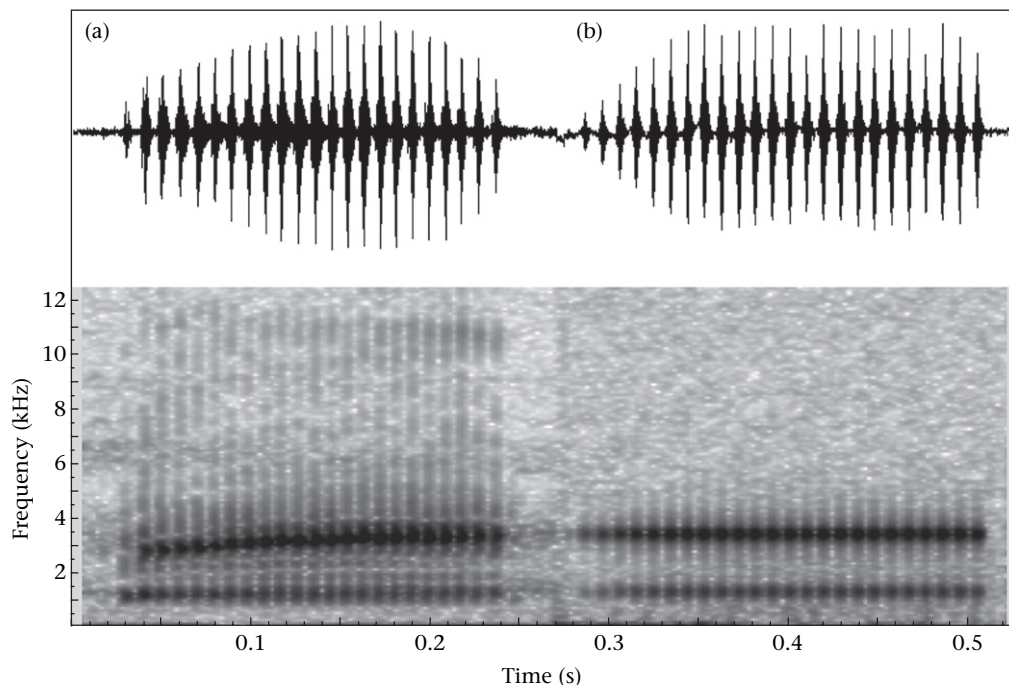

Figure 1. Exemplary waveforms (above) and sonagrams (below) of a natural advertisement call of *H. squirella* (a) and of a synthesized advertisement call (b) used in this study.

Table 1. Call properties of male advertisement calls of squirrel tree-frog from field recordings

Parameter	Mean	±1 SD	Range	N
Lower-frequency peak (kHz)	1.379	1.36	1.040–1.720	98
Higher-frequency peak (kHz)	3.344	2.44	2.780–4.000	98
Call rate (calls/min)	103	15.19	60–139	98
Call duration (s)	0.179	0.020	0.146–0.224	38
No. of pulses	20.6	2.0	16.9–24.4	38
Duty cycle (s)	0.538	0.074	0.370–0.684	38
Pulse rate (pulses/s)	115.2	4.25	108.4–125.2	38
Pulse duration (s)	0.008	0.00031	0.0080–0.0092	38
Intercall interval (s)	0.36	0.076	0.204–0.523	38

Data recorded from males in research enclosure ($N = 98$) and from the field ($N = 38$). All males were from Thistlethwaite Wildlife Management Area.

experiments, a high-frequency call possessed peak energy at 1.4 and 3.6 kHz, a medium-frequency call possessed dominant frequency peaks at 1.2 and 3.2 kHz, and a low-frequency call possessed dominant frequency peaks at 1.0 and 2.8 kHz. All signals were broadcast at 88 dB (fast RMS, C weighting, Radio Shack no. 33-2050 sound level meter), measured from the point of the females' release. Eighty-eight decibels was within the range of amplitudes experienced by females in the field. We systematically varied either frequency or call rate of a synthetic call while holding all other call parameters constant (e.g. pulse rate, call duration, etc.).

PHONOTAXIS EXPERIMENTS

Methods

Each trial consisted of placing a single female into an acoustically transparent restraining cage, equidistant (1.1 m) from two loudspeakers, forming a triangle such that the angle of the speakers relative to the female was 110°. We played back two synthesized calls antiphonally. After starting the playback, females were held in the restraining cage for a minimum of 2 min or until they stopped actively attempting to exit the cage. An observer seated approximately 2 m behind the focal female then removed the top of the restraining cage remotely by pulling a string that was run through an eye-bolt positioned over the cage. This allowed the female to leave the cage and approach one of the speakers. A female was scored as making a choice when she approached within 5 cm of the front of one of the loudspeakers. If a female failed to approach a speaker within 10 min, she was unlikely to make any choice, and the result was discarded from the data set. On a typical night we would have 10–12 amplectant females to test, and five to seven of these were usually responsive. Thus, each experiment required multiple nights of testing. After a trial, the female was removed and another trial was repeated with a new female. We alternated the side from which each call was broadcast between consecutive trials to control for position bias.

Female preferences were estimated by comparing responses for each stimulus against an equiprobable distribution (no preference) using the binomial test (one tailed). The SE for binomial distributions was calculated for the proportion of females choosing each stimulus (Zar 1984). Based on data from other North American hylid frogs (Gerhardt & Huber 2002), we predicted a priori that females would choose vocalizations that would be more energetically expensive for males to produce (faster call rates) or that would be indicative of larger males (lower-frequency calls); therefore, one-tailed tests were used.

Experiment 1: frequency

To evaluate female preferences for dominant frequency, we presented females with dichotomous choices among three different dominant frequencies covering a large portion of the range of frequencies produced by calling males (low-frequency call: 1.0 and 2.8 kHz; medium-frequency call: 1.2 and 3.2 kHz; high-frequency call: 1.4 and 3.6 kHz; Table 1). All calls were broadcast at a rate of 120 calls/min and 88 dB SPL relative to the point of release for the females.

Experiment 2: call rate

We presented females with three different stimulus pairs that differed in call rate: (1) 60 calls/min (cpm) versus 80 cpm, (2) 80 versus 120 cpm and (3) 120 versus 150 cpm. All calls were broadcast at 88 dB SPL at the point of female release and had dominant frequency peaks at 1.2 and 3.2 kHz (medium frequency). The call rates used in this study represent the range over which the majority of males can be found calling in natural choruses (Table 1). The low end of the calling rate that we tested (60 cpm) is likely to occur only rarely on nights with low-chorus density (<10 males) or an ambient air temperature that is near the lower threshold at which males call (19.0°C; personal observation). The highest call rate that we tested (150 cpm) is likely to occur only when a male detects an approaching female, whereby males often increase their call rates until the female either approaches and initiates amplexus or leaves the area (R. C. Taylor, personal observation).

Experiment 3: position bias control

As an overall control for side or speaker biases, we conducted an experiment where females were given a choice between two identical calls broadcast from the same speakers used in the other experiments. We did not switch the position of the signal or speakers between trials to control for potentially subtle differences in the recording quality between channels or the differences in loudspeaker reproduction. Calls presented to females in this experiment had the following characteristics: medium frequency = 1.2 and 3.2 kHz peaks, call rate = 120 cpm, SPL = 88 dB.

Results

Experiment 1: frequency

Females consistently preferred the call with the lower dominant frequency when one of the calls presented was

of high dominant frequency. When we presented females with a choice between a high-frequency and low-frequency call, females chose the low-frequency signal significantly more often ($N = 19, P < 0.0001$; Fig. 2). When presented with high-frequency versus medium-frequency calls, females showed a preference for the medium-frequency calls significantly more often ($N = 20$, $P < 0.0001$; Fig. 2). However, when we presented females with a medium- versus low-frequency call, females showed no preference for either call ($N = 19, P = 0.500$; Fig. 2).

Experiment 2: call rate

When we presented females with a choice of 60 versus 80 cpm, they chose 80 cpm significantly more often ($N = 18, P = 0.0006$; Fig. 3). When females were presented with a choice of 80 versus 120 cpm, they chose 120 cpm significantly more often ($N = 21$, $P = 0.0007$; Fig. 3). When presented with calls of 120 versus 150 cpm, females chose 150 cpm significantly more often ($N = 21$, $P = 0.0392$). In the 120 versus 150 cpm stimulus pair, however, females did not discriminate among the calls as strongly as the other stimulus pairs (Fig. 3).

Experiment 3: position bias control

We tested for side biases by giving females a choice between two identical calls. Females did not show a significant preference for calls produced from either side of the test arena (left side: eight females, right side: nine females, $P = 0.500$). This suggests that female responses in the previous experiments were unlikely to be influenced by position biases or subtle differences in signal quality between the two channels (speakers).
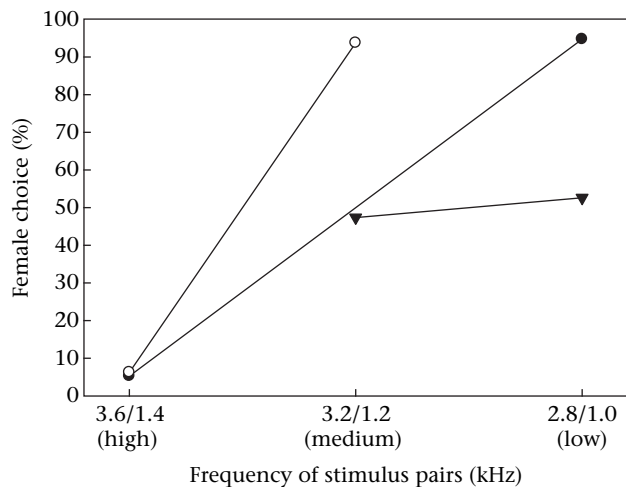

Figure 3. Preference function of female choice (percentage choosing a particular stimulus) for call rate (calls/min). The lines connecting each of two points represent the percentage of females selecting one alternative stimulus over another in particular paired stimulus treatments. Direct statistical comparisons were not made among the three stimulus pairs; rather, the preferences expressed by females indicate female preferences over the range of call rate variation typically found among males in a natural chorus.

VISUAL STIMULUS EXPERIMENTS

Methods

For the visual preference experiments, we followed the general procedures outlined for the phonotaxis experiments; protocols specific to these experiments are outlined below. It was necessary to collect frogs in the enclosure before testing by illuminating them with a headlamp. Therefore, after collection, we placed the cups containing females into an insulated light-safe box (cooler) so that the females were in total darkness. We left the females in total darkness for a minimum of 1 h before testing so that their eyes would be dark-adapted during experiments (Cornell & Hailman 1984; Fain et al. 2001). The moon was not high enough in the sky to introduce light directly into the enclosure during any experiment, therefore, no strong shadows were cast by the moon that would create differential illuminations across the arena. The light meter (Science & Mechanics Light Meter Model 102) that we used for measurements was only capable of measuring light levels down to 10^{-3} lx. On each testing night, the light meter did not register, indicating that the light level was $<10^{-3}$ lx. This light level is commensurate with conditions under which squirrel treefrogs normally breed (Buchanan 1992).

Experiment 1: the influence of visual cues on female choice

Each trial consisted of placing a single female into a restraining cage, equidistant (0.8 m) from two loudspeakers forming a triangle such that the angle of the speakers relative to the female was 90°. We broadcast the same call antiphonally from each speaker. The synthesized call presented to the females consisted of two frequency

Figure 2. Preference function of female choice (percentage choosing a particular stimulus) for call frequency (kHz). The lines connecting each of two points represent the percentage of females selecting one alternative stimulus over another in particular paired stimulus treatments. Direct statistical comparisons were not made among the three stimulus pairs; rather, the preferences expressed by females indicate female preferences over the range of frequency variation typically found among calling males.
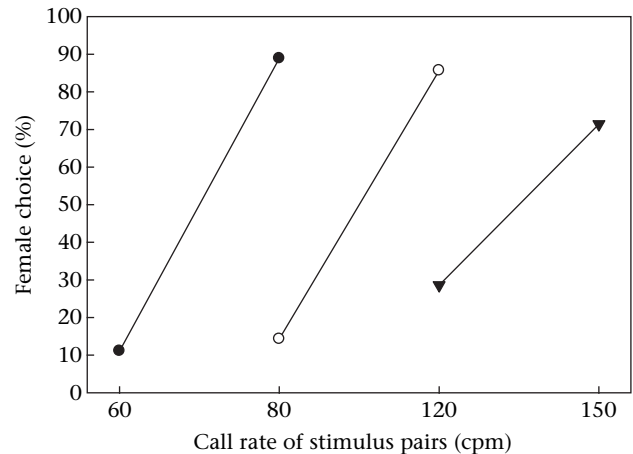
peaks (1.2 and 3.2 kHz) and was broadcast at a rate of 120 cpm and 88 dB SPL measured from the females' release point. We previously showed that this call is attractive to females (Figs 2 and 3). By playing the same call from each speaker, we removed the females' ability to make a phonotactic response based on differing call properties. The restraining cage was constructed by placing black window screening between the top and bottom portions of a 9-cm plastic petri dish; it was reasonably transparent to both acoustic and visual stimuli.

A visual stimulus was provided to the females by placing a painted, plaster model of a calling male in front of one of the speakers. Models were placed at a 90° angle to females and parallel to the front edge of the speaker. In two-model tests, models at different speakers faced each other. The model used in this experiment was designed to represent a male with large labial and lateral stripes (stripe size = 5; Buchanan 1994). All models used in the visual stimulus experiments were created using the same mould and were identical in size and shape. Models were painted with enamel paints. Reflectance spectra of labial and lateral stripes, dorsum, and deflated vocal sacs of models and real frogs are presented in Fig. 4. Spectra from live frogs are averages of spectra made from three individuals. Because of the dynamic polychromatic coloration of squirrel treefrog adults (Buchanan 1994), no attempt was made to match model frog colour to living frog colour exactly. All spectra were generated by holding the spectrometer fibre optic probe 1−2 mm above the surface being sampled at a 45° angle to the surface and light source. Spectra of deflated vocal sacs of live frogs were made from photographs and may not be accurate representations of actual reflected spectra, although they appeared normal to the researchers. Spectra were generated using a StellarNet EPP2000C reflectance microspectrometer with fibre optic probe (0.5 nm resolution) calibrated with a 97% reflectance Halon standard using SpectraWiz software. The light source was a Phillips 50 W halogen lamp (Fig. 4) illuminating the subject from directly above.

We attached a portion of a Trojan® brand latex condom to the vocal sac region of the models and inflated it with air synchronously with the call being broadcast from the speaker, thereby mimicking the movement of the vocal sac of a calling male. When inflated, the condom appeared to mimic the translucent quality of a live male's inflated vocal sac. Inflation was achieved by a hand squeeze-bulb connected to a thin rubber tube attached to the back of the model.

For each trial, we placed a male model in front of one speaker (the back edge of model was within 1 cm from the front of the speaker) and no model in front of the other speaker. We then started the call playbacks. Once the playback was started, we introduced a female into the restraining cage and began inflating the model's vocal sac synchronously with the call rate. The female was allowed to remain in the cage for a minimum of 2 min. Before release, we required that the female had oriented her head towards both sides of the arena at least once so that she had the opportunity to gain visual information from the source of both vocal calls. The female was then released from the restraining cage only when she was oriented in
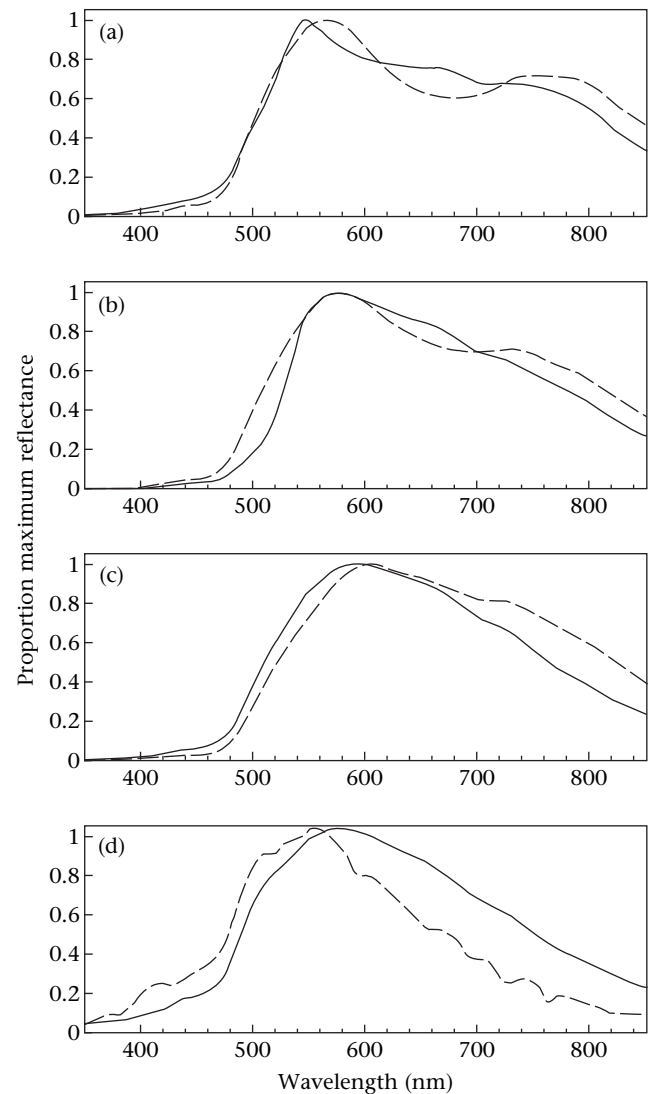

Figure 4. Average reflectance spectra (proportion maximum reflectance at each wavelength, nm) generated from models and frogs. (a) Reflectance spectra of green dorsum of model frog (solid lines) and green dorsum of live frog (dashed lines). (b) Reflectance spectra of labial and lateral stripes of model (solid) and live frog (dashed). (c) Reflectance spectra of vocal sac of model (solid) and live frog (dashed). (d) Reflectance spectra of source light used to generate spectra (solid) and sunlight (dashed).

a manner whereby she was facing within a 20° arc of either side of the midpoint of the two stimuli. This criterion minimized the possibility that the female's initial orientation, upon release, would influence her direction of travel.

Once a female met the criteria, we removed the top of the restraining cage remotely and observed the female through a hole in a sheet of black polyethylene plastic using an IR viewer. A female was scored as making a choice when she approached to within 5 cm of the front of one of the loudspeakers. To avoid position bias, we alternated the side on which the model was presented between each trial. If a female failed to make a choice after 10 min, we discarded that result from the data set.

This experiment was designed to test the hypothesis that females use visual information when assessing potential mates. It did not, however, reveal what particular visual characteristics of a calling male might be salient features for attraction. Therefore, experiments 2 and 3 were designed to test for specific visual characters that might be important to females.

Experiment 2: the influence of the vocal sac on female choice

The protocol for experiment 2 was identical to that of the first experiment. However, instead of giving females a choice with a model on only one side, the choice was between a dynamic model (vocal sac inflating synchronously with the call) and a static model (deflated, nonmoving vocal sac). We alternated the sides on which the models were presented between trials.

Experiment 3: the influence of lateral stripe size on female choice

Experiment 3 was identical to the second experiment except that the visual cues presented to the females were models that differed in the size of their labial and lateral stripes. Females were given a choice between a dynamic model with a conspicuous stripe painted on the upper lip and the side of the torso (area approximately 57 mm^2, equivalent to stripe size = 5; Buchanan 1994) and a dynamic model with a very thin stripe painted on the upper lip and side of the torso (area approximately 7.5 mm^2; equivalent to stripe size = 1: Buchanan 1994). The areas of the stripe sizes are given as approximations because the jagged edges of the stripe borders on the models (as occurs in live frogs) prevented an exact linear measurement. The stripe sizes on the models were within the range of variation found on males in nature (Buchanan 1994).

Results

Experiment 1: the influence of visual cues on female choice

In this experiment, females chose the speaker with the dynamic model significantly more often than the speaker lacking the model (Table 2). Five of the 18 females (27.7%) that chose the side with the model attempted to initiate amplexus with the model by touching the vocal sac and then climbing on the model's back or presenting their posterior end to the model.

Experiment 2: the influence of the vocal sac on female choice

When females were presented with dynamic or static models along with identical calls from each speaker there was no significant difference in the preferences of females for dynamic or static models. There was a slight trend towards females choosing the dynamic model over the static model (Table 2). Two of the 13 females (15.38%) responding to the dynamic model attempted to initiate amplexus with the model. None of the seven females that

Table 2. Female choice for visual cues

Choice		P	SE
Model	No model		
78.3 (18)	21.7 (5)	0.0053	0.089
Dynamic model (DM)	Static model (SM)		
65.0 (13)	35.0 (7)	0.132	0.110
Large stripe (DM)	Small stripe (DM)		
73.9 (17)	26.1 (6)	0.017	0.095

Percentage of females choosing between alternative stimuli. Numbers in parentheses are the number of females choosing each stimulus. The binomial SE is reported for the proportion. Dynamic model (DM): model with vocal sac inflated synchronously with call; static model (SM): model with a vocal sac that remained deflated during trials; cpm: calls per min.

responded to the static model attempted to initiate amplexus.

Experiment 3: the influence of lateral stripe size on female choice

Females were presented with two visual stimuli in this experiment. Dynamic models were used in front of each loudspeaker on each side of the arena, but the models differed in the sizes of their labial and lateral stripes. Females chose the model with a larger stripe significantly more often over the smaller-striped alternative (Table 2). Two of the 17 females (11.76%) that responded to the large-striped model attempted to initiate amplexus. None of the six females responding to the small-striped model attempted to initiate amplexus.

DISCUSSION

In our experiments, females discriminated against high-frequency calls in favour of medium- or low-frequency alternatives but females were equally attracted to low- and medium-frequency calls. This pattern suggests that females discriminate against small males. Because small males have higher-frequency advertisement calls, such a female preference should produce a large-male mating advantage. However, Buchanan (1993b) found no large-male mating advantages in natural or experimental choruses. This suggests that alternative male strategies may be subverting female choices or that other factors, such as the use of visual cues, are likely to influence female mate choice.

Females also expressed strong preferences for faster call rates. Mating preferences by females of a number of species of anurans show that females often prefer calls that are more energetically expensive to produce (Gerhardt & Huber 2002). If other properties are held equal, as they were in this experiment, then increasing call rate produces more total energy. Male squirrel treefrogs participate in some of the most energetically expensive calling thus studied among anurans (Prestwich et al. 1989). It is not clear from our design if female preferences for call rate were indicative of a preference for more total energy or the faster call rate alone. Our results with females of

squirrel treefrogs are consistent with the pattern of prefer-ence for faster call rates shown by some other species (Gerhardt et al. 1996; Gerhardt & Huber 2002. Additional experiments will be necessary, however, to determine whether total energy or call rate is more important in elic-iting female phonotactic responses.

Despite strong female preferences for more energetically expensive calls in simple experiments, females do not always choose males giving the most expensive calls under natural conditions. Schwartz et al. (2001) showed that in simple two-stimulus tests, female grey treefrogs showed strong preferences for male vocal calls of longer duration, and discriminated against shorter-duration calls. In these experiments, females even discriminated among calls that differed in as few as two call pulses. Under more natural conditions, however, females discriminated against only the very shortest calls, and thus call duration was not a strong predictor of male mating success (Schwartz et al. 2001).

Choruses are often noisy and acoustically complex environments. Call overlap and background noise can degrade signals or interfere with signal detection and make it difficult for females to assess individual males within a chorus (Gerhardt & Klump 1988; Schwartz 1993; Wollerman 1999). One explanation for the reduction in the expression of female preferences for call properties under natural conditions may stem from this problem. If females attempt to express preferences for certain call properties of males, they may be unable to do so effi-ciently in many chorus situations. Another explanation may stem from interactive effects of different call proper-ties. For example, small males producing calls with high dominant frequencies may be able to increase their rela-tive attractiveness by calling at higher rates (Smith & Roberts 2003).

Vocalizations are sufficient for female attraction. How-ever, our results show that females of squirrel treefrogs also use visual information to assess potential mates when such information is available. In the first visual cue experiment, females chose the call with the model significantly more often than the same call lacking a model. This suggests that, given two calls of equal attractiveness, females are likely to choose a male that is visually accessible. This is a situation that females com-monly encounter under natural field conditions. The habitat (woodland ponds and ditches) where choruses occur is replete with emergent aquatic vegetation and tree branches, creating a structurally complex environment. Females typically enter a chorus and appear to assess a local group of males within the larger chorus (R. C. Taylor, personal observation). Of the several males that the female may be able to detect acoustically, she is likely to have visual information available about only a subset of these males. Approximately 15–20% of males in a chorus will call from a site that is visually unobstructed by vegetation, with the remainder calling from under the leaves of plants, although this ratio seems to depend heavily on ambient light levels (R. C. Taylor, personal observation). The results from experiment 1 suggest that those males that make themselves visually accessible may gain a mating advantage over males that remain hidden in the vegetation. This advantage may occur because visibil-ity may improve a male's detectability, discriminability, or memorability (Rowe 1999) to females.

There may also be a cost to males who make themselves most visible; they may increase their probability of de-tection by predators. We commonly observed numerous snakes (Agkistrodon piscivorous, Nerodia fasciata, Nerodia rhombifer, and Nerodia erythrogaster), mud turtles, Kinoster-non subrubrum, and giant water bugs (Lethocerus sp.) prey-ing on frogs. In addition, on nights during and around a full moon and in light-polluted areas, males either do not call or call only from deep within vegetation. Detec-tion by predators may explain why only a relatively small proportion of males call from sites unobstructed by vegetation.

Although the results of the first visual experiment showed that females do use visual cues, they did not yield any information about what particular characteristics of a male may be detectable or attractive to a female. Experiment 2 tested whether females express a preference for a moving vocal sac that could be an important visual cue to females for two reasons. First, anuran amphibians are visually sensitive to movement (Lettvin et al. 1968), and thus the movement of the vocal sac may be easy for a female to detect under low-illumination conditions. Squirrel treefrogs are capable of detecting moving prey us-ing vision alone at nocturnal illuminations typical of those experienced during chorusing (Buchanan 1998). Second, satellite behaviour in the squirrel treefrog is com-mon (Buchanan 1993b) and females who approach calling males encounter satellite males with a relatively high fre-quency. Visually discriminating the calling male from the satellites would then increase the probability that the fe-male would mate with the male to which she was origi-nally attracted.

More females chose the side with the dynamic model over the static model, but the difference was not signifi-cant. Given the visual sensitivity that anurans express towards movement, this result was somewhat surprising. Rosenthal et al. (2004) showed that females of P. pustulosus show preferences for inflating vocal sacs, but our experi-ment suggests that female squirrel treefrogs either have difficulty detecting the translucent vocal sac under noc-turnal conditions or they may not rely on it as an impor-tant visual cue.

In experiment 3, females chose the side with a model possessing a large stripe significantly more often than the side with a model possessing a small stripe. This result suggests that stripe size may be an important visual cue that females use to locate or assess males. In natural populations, the stripe size and chromatic reflectance is variable in both sexes, but males show larger and/or more chromatically saturated (more yellow) stripes (Buchanan 1994). In our experiment, chromatic reflectance of the stripes was held constant and the stripe sizes on the models represented the two ends of the size range over which variation occurs in live males.

The lateral stripe is concealed by the fore- and hindlimbs when frogs are in daytime retreats (R. C. Taylor, personal observation) or in a water conservation posture. When males are calling, the stripe is displayed prominently as the

inflated trunk of the male raises the stripe away from the substrate, stretches it, and forces the hindlimbs to a more posterior position. Our results suggest that, all else being equal, males who possess a large stripe are more likely to be chosen by a female than males possessing a diminutive stripe.

One possible reason for females to select males with larger stripes may be that stripes provide some information about male quality; males that are in better condition may invest more energy into pigmentation for stripe production or some males may have greater access to pigment-producing chemicals (Hamilton & Zuk 1982). Stripe size does not appear to be labile within individuals, but laboratory experiments have shown that the yellow stripe pigmentation may be indicative of recent feeding history (R. C. Taylor, personal observation). The mechanism controlling stripe size or chromatic saturation, however, is currently unknown. Therefore, the results of this experiment do not yield any evidence to support this hypothesis. A second and perhaps more likely explanation may stem from detectability. Unlike the vocal sac, the lateral stripe provides contrast in its position adjacent to the darker dorsum coloration (see Figure 1 in Buchanan 1994). Females may be able to detect a high-contrast stripe more easily under low-illumination conditions. In addition to detection, Ryan & Keddy-Hector (1992) and Jansson & Enquist (2003) suggested that receivers respond more strongly to bright or colourful stimuli. If females of squirrel treefrogs have an inherent sensory bias for contrast or colour, then this would be sufficient to explain the attractiveness of a large and/or highly colour-saturated stripe (Ryan et al. 1990). It is currently unknown, however, whether or not colour vision functions at the low nocturnal illuminations at which these frogs are active.

The use of the models in our experiments had several advantages over using live males as a visual stimulus. Live males in reproductive condition would have been likely to call, rendering it impossible to determine whether females were choosing auditory or visual cues. The models also allowed us to control for variability in body size and chromatic characteristics between different potential visual stimuli. The models also eliminated any unique olfactory cues that would be produced by live males, and eliminated any uncontrolled movements. Approximately 12% of all females tested (11 of 89) attempted to initiate amplexus with the models. Although the models were not perfect representations of live males, the behaviour of the females suggests that the models were accurate enough to invoke realistic behavioural responses.

Multimodal signals may have evolved to transmit the same information (redundant signals) or different information (nonredundant) (Partan & Marler 1999). The unimodal components of redundant signals should produce equal responses in receivers. In nonredundant signals, one component may modulate the receiver response to the other component (Partan & Marler 1999, 2005). We did not test female responses to the frog model without a call, thus our data do not yet allow for classification of this system into the broad framework of redundant versus nonredundant signalling. The chorus environment where these frogs breed is acoustically noisy. Thus, it seems likely that multimodal signalling evolved in this system as an efficacy trade-off (Hebets & Papaj 2005), whereby the auditory signal is detectable from a relatively long distance and the visual cue increases the ability of a female to localize a particular male. The ability of a female to localize a male quickly through the use of visual cues may also be selected for in this system where predation rates are relatively high, but the costs of visual signalling in this species need to be explored further.

Anuran amphibians have long been considered to be auditory specialists with regard to social signalling. However, our data provide additional evidence (Summers et al. 1999; Hödl & Amézquita 2001; Narins et al. 2003; Amézquita & Hödl 2004; Rosenthal et al. 2004; Narins et al. 2005) that visual cues play a role in the breeding system of anuran amphibians, even those that are only active nocturnally. Specifically, our data show that visual cues are especially likely to influence the mate choice of female squirrel treefrogs when they encounter vocalizations of approximately equal attractiveness. We argue that providing a broader range of information to females during choice experiments is likely to provide a better understanding of patterns of mating success and the process by which female choice can drive the evolution of multicomponent sexual displays.

References

Amézquita, A. & Hödl, W. 2004. How, when, and where to perform visual displays: the case of the Amazonian frog, *Hyla parviceps*. *Herpetologica*, **60**, 420−429.

Andersson, M. 1982. Female choice selects for extreme tail length in a widowbird. *Nature*, **299**, 818−820.

Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.

Basolo, A. 1990. Female preference for male sword length in the swordtail *Xiphophorus helleri* (Pisces: Poeciliidae). *Animal Behaviour*, **40**, 332–338.

Buchanan, B. W. 1992. Bimodal nocturnal activity pattern of *Hyla squirella*. *Journal of Herpetology*, **26**, 521–522.

Buchanan, B. W. 1993a. Effects of enhanced lighting on the behaviour of nocturnal frogs. *Animal Behaviour*, **45**, 893–899.

Buchanan, B. W. 1993b. The influence of the competitive environment on the expression of satellite behavior in anuran amphibians. Ph.D. thesis, University of Southwestern Louisiana, Lafayette.

Buchanan, B. W. 1994. Sexual dimorphism in *Hyla squirella*: chromatic and pattern variation between the sexes. *Copeia*, **1994**, 797–802.

Buchanan, B. W. 1998. Low-illumination prey detection by squirrel treefrogs. *Journal of Herpetology*, **32**, 270–274.

Candolin, U. 2003. The use of multiple cues in mate choice. *Biological Review*, **78**, 575–595.

Cornell, E. A. & Hailman, J. P. 1984. Pupillary responses of two *Rana pipiens*: complex anuran species. *Herpetologica*, **40**, 356–366.

Crapon de Caprona, M. & Ryan, M. J. 1990. Conspecific mate recognition in swordtails, *Xiphophorus nigrensis* and *X. pygmaeus* (Poeciliidae): olfactory and visual cues. *Animal Behaviour*, **39**, 290–296.

Elias, D. O., Hebets, E. A., Hoy, R. R. & Mason, A. C. 2005. Seismic signals are crucial for male mating success in a visual specialist jumping spider (Araneae: Salticidae). *Animal Behaviour*, **69**, 931–938.

Fain, G. L., Matthews, H. R., Cornwall, M. C. & Koutalos, Y. 2001. Adaptation in vertebrate photoreceptors. *Physiological Reviews*, **81**, 117–151.

Gerhardt, H. C. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. *Animal Behaviour*, **42**, 615–635.

Gerhardt, H. C. & Huber, F. 2002. *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. Chicago, Illinois: Chicago University Press.

Gerhardt, H. C. & Klump, G. M. 1988. Masking of acoustic signals by the chorus background noise in the green tree frog: a limitation on mate choice. *Animal Behaviour*, **36**, 1247–1249.

Gerhardt, H. C., Dyson, M. L. & Tanner, S. D. 1996. Dynamic acoustic properties of the advertisement calls of gray treefrogs: patterns of variability and female choice. *Behavioral Ecology*, **7**, 7–18.

Haddad, C. F. B. & Giaretta, A. A. 1999. Visual and acoustic communication in the Brazilian torrent frog, *Hylodes asper* (Anura: Leptodactylidae). *Herpetologica*, **55**, 324–333.

Hamilton, W. D. & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, **218**, 284–387.

Hasselquist, D. S., Bensch, S. & von Schantz, T. 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature*, **381**, 229–232.

Hebets, E. A. & Papaj, D. R. 2005. Complex signal function: developing a framework of testable hypotheses. *Behavioral Ecology and Sociobiology*, **57**, 197–214.

Hirschman, W. & Hödl, W. 2006. Visual signaling in *Phrynobatrachus krefftii* Boulenger, 1909 (Anura: Ranidae). *Herpetologica*, **62**, 18–27.

Hödl, W. & Amézquita, A. 2001. Visual signaling in anuran amphibians. In: *Anuran Communication* (Ed. by M. J. Ryan), pp. 121–141. Washington, D.C: Smithsonian Institution Press.

Houde, A. E. 1987. Mate choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution*, **41**, 1–10.

Jansson, L. & Enquist, M. 2003. Receiver bias for colourful signals. *Animal Behaviour*, **66**, 965–971.

Kodric-Brown, A. & Nicoletto, P. F. 2001. Female choice in the guppy (*Poecilia reticulata*): the interaction between male color and display. *Behavioral Ecology and Sociobiology*, **50**, 346–351.

Larsen, L. O. & Pedersen, J. N. 1982. The snapping response of the toad, *Bufo bufo*, towards prey dummies at very low light intensities. *Amphibia-Reptilia*, **2**, 321–327.

Lettvin, J. Y., Maturana, H. R., McCulloch, W. S. & Pitts, W. H. 1968. What the frog's eye tells the frog's brain. In: *The Mind: Biological Approaches to its Functions* (Ed. by W. C. Corning & M. Balaban), pp. 233–258. New York: J. Wiley.

Lindquist, E. D. & Hetherington, T. E. 1996. Field studies on visual and acoustic signaling in the 'earless' Panamanian golden frog, *Atelopus zeteki*. *Journal of Herpetology*, **30**, 347–354.

Lynch, K. S., Crews, D., Ryan, M. J. & Wilczynski, W. 2006. Hormonal state influences aspects of female choice in the túngara frog (*Physalaemus pustulosus*). *Hormones and Behavior*, **49**, 450–457.

Narins, P. M., Hodl, W. & Grabul, D. S. 2003. Bimodal signal requisite for agonistic behavior in a dart-poison frog, *Epipedobates femoralis*. *Proceedings of the National Academy of the Sciences, U.S.A.*, **100**, 577–580.

Narins, P. M., Grabul, K. K. S., Gaucher, P. & Hodl, W. 2005. Cross-modal integration in a dart-poison frog. *Proceedings of the National Academy of the Sciences, U.S.A.*, **102**, 2425–2429.

Partan, S. R. & Marler, P. 1999. Communication goes multimodal. *Science*, **283**, 1272–1273.

Partan, S. R. & Marler, P. 2005. Issues in the classification of multimodal communication signals. *American Naturalist*, **166**, 231–245.

Petrie, M. & Halliday, T. 1994. Experimental and natural changes in the peacock's (*Pavo cristatus*) train can affect mating success. *Behavioral Ecology and Sociobiology*, **35**, 213–217.

Prestwich, K. N., Brugger, K. E. & Topping, M. 1989. Energy and communication in three species of hylid frogs: power input, power output and efficiency. *Journal of Experimental Biology*, **144**, 53–80.

Rosenthal, G. G., Rand, A. S. & Ryan, M. J. 2004. The vocal sac as a visual cue in anuran communication: an experimental analysis using video playback. *Animal Behaviour*, **68**, 55–58.

Rowe, C. 1999. Receiver psychology and the evolution of multicomponent signals. *Animal Behaviour*, **58**, 921–931.

Ryan, M. J. (Ed). 2001. *Anuran Communication*. Washington, D.C: Smithsonian Institution Press.

Ryan, M. J. & Keddy-Hector, A. 1992. Direction patterns of female mate choice and the role of sensory biases. *American Naturalist*, **139**, 4–35.

Ryan, M. J., Fox, J. H., Wilczynski, W. & Rand, A. S. 1990. Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature*, **343**, 66–67.

Schwartz, J. J. 1993. Male calling behavior, female discrimination and acoustic interference in the neotropical treefrog *Hyla microcephala* under realistic acoustic conditions. *Behavioral Ecology and Sociobiology*, **32**, 401–414.

Schwartz, J. J., Buchanan, B. W. & Gerhardt, H. C. 2001. Female mate choice in the gray treefrog (*Hyla versicolor*) in three experimental environments. *Behavioral Ecology and Sociobiology*, **49**, 443–455.

Smith, M. J. & Roberts, J. D. 2003. Call structure may affect male mating success in the quacking frog *Crinia georgiana* (Anura: Myobatrachidae). *Behavioral Ecology and Sociobiology*, **53**, 221–226.

Summers, K., Symula, R., Clough, M. & Cronin, T. 1999. Visual mate choice in poison frogs. *Proceedings of the Royal Society of London, Series B*, **266**, 1–5.

Telford, S. D., Dyson, M. L. & Passmore, N. I. 1989. Mate choice occurs only in small choruses of painted reed frogs *Hyperolius marmoratus. Bioacoustics*, **2**, 47–53.

Wells, K. D. 1980. Social behavior and communication of a dendrobatid frog (*Colostethus trinitatis*). *Herpetologica*, **36**, 189–199.

Wollerman, L. 1999. Acoustic interference limits call detection in a neotropical frog, *Hyla ebraccata. Animal Behaviour*, **57**, 529–536.

Zar, J. H. 1984. *Biostatistical Analysis*. 2nd edn. Englewood Cliffs, New Jersey: Prentice-Hall.