



Neighbour–stranger discrimination by territorial male bullfrogs (*Rana catesbeiana*): II. Perceptual basis

MARK A. BEE & H. CARL GERHARDT

Division of Biological Sciences, University of Missouri–Columbia

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Territorial animals commonly display lower levels of aggression towards familiar neighbours in familiar locations than towards neighbours in unfamiliar locations and towards unfamiliar individuals. A combination of acoustic signals and spatial cues mediates this form of social recognition in a variety of animals, including the North American bullfrog, *Rana catesbeiana*. In this study, we conducted two field playback experiments to investigate the perceptual basis of neighbour–stranger discrimination in bullfrogs. In a discrimination test following habituation training, a change of 10% in the fundamental frequency or 180° in the broadcast location of a synthetic bullfrog advertisement call elicited significant recovery of habituated aggressive responses. Hence, male bullfrogs can learn about an individually distinct property of acoustic signals and the signal's location of origin by repeatedly hearing the signal from a particular location. This study represents the first direct test of the hypothesis that territorial residents can learn about a specific property of a neighbour's acoustic signals and the location from which these are normally produced as a result of repeated exposures to the signal. We suggest that reduced aggression between territorial neighbours could be partially mediated by habituation to a neighbour's signals and location in bullfrogs.

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Many territorial animals display a form of social recognition referred to as the 'dear enemy phenomenon' (Wilson 1975), in which they discriminate between strangers and adjacent territorial neighbours, which are usually also associated with particular locations (reviews in Ydenberg et al. 1988; Temeles 1994). By recognizing established neighbours, territory owners presumably avoid the costs incurred in repeated aggressive interactions with conspecifics that pose little threat to territory ownership. Most previous studies have used field playback experiments to investigate vocally mediated neighbour–stranger discrimination in territorial songbirds (reviews in Falls 1982; Lambrechts & Dhondt 1995; Stoddard 1996). In these studies, residents typically display reduced levels of aggression to a neighbour's acoustic signals broadcast from the direction of the neighbour's territory, but respond aggressively to signals of familiar neighbours from 'unfamiliar' or 'incorrect' locations and to those of strangers. Beer (1970) first pointed out that discrimination between neighbours and strangers in the neighbour's territory could result if animals simply dis-

criminate between familiar (neighbours) and unfamiliar (strangers) conspecifics. Aggressive responses to neighbours (or their signals) outside their usual territories are commonly regarded as evidence that neighbours are recognized as particular individuals (e.g. Beer 1970; Falls & Brooks 1975; Wiley & Wiley 1977; Myrberg & Riggio 1985; Godard 1991; Stoddard et al. 1991; Godard & Wiley 1995).

In a previous field playback experiment, Davis (1987) demonstrated that territorial males of the North American bullfrog, *Rana catesbeiana* (Anura, Ranidae), responded more aggressively to the advertisement calls of strangers than to those of neighbours when both kinds of calls were broadcast from the neighbour's territory. Males also responded more aggressively to a neighbour's advertisement calls when broadcast from a novel location than when broadcast from the neighbour's territory. As in territorial songbirds (Falls 1982; Lambrechts & Dhondt 1995; Stoddard 1996), male bullfrogs learn about the acoustic signals of their neighbours and behaviourally associate these signals with specific locations in the breeding aggregation.

In a companion paper (Bee & Gerhardt 2001a), we examine the acoustic basis of vocally mediated neighbour–stranger discrimination in bullfrogs. Based on our analyses, we hypothesized that patterns of variation

Correspondence and present address: M. Bee, Department of Biology, Carl von Ossietzky University, D-26111 Oldenburg, Germany (email: mabf79@mizzou.edu). H. C. Gerhardt is at the Division of Biological Sciences, 105 Tucker Hall, University of Missouri–Columbia, Columbia, MO 65211-7400, U.S.A.

in fundamental frequency, the correlated property of dominant frequency, or both, might form a basis for discriminating between the calls of different individuals. Here, we report results from two field playback experiments that investigated the perceptual basis of neighbour–stranger discrimination in bullfrogs. Using a habituation/discrimination procedure, we asked whether repeated broadcasts of the advertisement calls of a simulated territorial neighbour would result in aggressive response decrements that were specific to both the fundamental frequency of advertisement calls and the location from which these signals were broadcast.

METHODS

Our field study was conducted between May and August, 1996–1999, in central Missouri at the Little Dixie Conservation Area (Callaway Co.) and the Thomas Baskett Wildlife Area (Boone Co.). We tested territorial male bullfrogs that were individually marked for identification using a combination of toe clips and waistbands. Additional details concerning our field sites and marking methods can be found elsewhere (Bee & Gerhardt 2001a,b).

The Study System

The bullfrog mating system is a resource defence polygyny. During the summer breeding season, males establish territories that females use as oviposition sites (Emlen 1968, 1976; Howard 1978). In our study populations, some individual males occupied the same territory for more than 4 weeks (see also Haas 1977), and adjacent neighbours sometimes shared a common territorial boundary for more than 2 weeks. Territorial residents exclude other conspecific males from calling within their territory using stereotyped aggressive movements, splash displays, and advertisement and encounter calls (Emlen 1968; Wiewandt 1969; Davis 1987; see Figure 1 in Bee & Gerhardt, 2001b). Agonistic interactions sometimes escalate to intense bouts of wrestling. Encounter calls are given when other bullfrogs trespass on a male's territory or during aggressive interactions between males, and they are often produced prior to physical fighting. Previous field playback experiments have established that territorial males respond aggressively with encounter calls, aggressive movements and approaches towards speakers broadcasting either prerecorded natural calls (Emlen 1968; Wiewandt 1969; Haas 1977; Davis 1987) or synthetic calls (Davis 1988; Bee & Gerhardt, 2001b).

General Playback Protocol

We conducted two field playback experiments under ambient light conditions between 2200 and 0700 hours Central Daylight Time. Our protocol in experiments 1 and 2 was based on the habituation/discrimination paradigm. In this procedure, a response-eliciting stimulus is presented during an initial habituation training phase until a response decrement occurs. Following habituation

training, a novel test stimulus is presented during a discrimination phase. Significant recovery of the habituated response during the discrimination phase indicates that animals can discriminate between the training and test stimuli. We chose the habituation/discrimination procedure for several reasons. First, this technique takes advantage of responses in an animal's natural behavioural repertoire and is therefore amenable to field studies that test behaviour in a natural setting (Halpin 1986). Second, the technique is widely used to assess discrimination capabilities in animals and has been used to examine aspects of individual recognition and discrimination in a number of species (e.g. Johnston & Jernigan 1994; Swaisgood et al. 1999). Finally, habituation probably plays some role as a mechanism underlying reduced aggression between neighbouring territorial animals (Brooks & Falls 1975a; Wiley & Wiley 1977; Peeke 1984; Shettleworth 1998). We recently demonstrated that the territorial aggressive response of male bullfrogs shows several characteristics of habituation in response to repeated broadcasts of a simulated new neighbour (Bee 2001; Bee & Gerhardt 2001b).

We used GoldWave 4.02 sound software to broadcast synthetic bullfrog advertisement calls via digital-to-analogue outputs from portable computers (Samsung SENS 800 and Dell Inspiron 3500) through 10-inch (25.4 cm) Optimus or Realistic speakers using Nagra DH or Rockford Fosgate 2.6× amplifiers. Speakers were mounted in wooden boxes and floated on styrofoam platforms covered in black plastic. The frequency response of the playback system was flat (± 4 dB) over the range of frequencies in our stimuli. At least 20 min prior to the beginning of a playback test, speakers were placed at a distance of 6 m from the subject along the pond bank. Neighbouring territorial male bullfrogs are commonly spaced between 3 and 16 m apart (Emlen 1968, 1976). We preferentially tested males that did not have a nearest neighbour within 10 m in at least one direction along the pond bank to avoid interference from other territorial males. The directionality of the speakers prevented the elicitation of aggressive responses from nearby neighbours. The speaker used during the habituation phase was always placed in the direction of the subject's most distant neighbour. Neighbouring males were never displaced in order to place the speaker. If there were no nearby neighbours (e.g. <15–20 m away), we determined the position of the habituation phase speaker relative to the subject (i.e. left or right) by flipping a coin. We began playback tests 10–20 min after the subjects resumed normal calling behaviour following speaker placement. Depending on the particular experiment, we broadcast stimuli at sound pressure levels (SPLs) of 87 or 81 dB (re 20 μ Pa, fast RMS, C-weighted) measured at a distance of 1 m. These values were selected to reflect the upper and lower end of the range of variation in the SPL of natural calls (Megela-Simmons 1984; M. A. Bee, unpublished data). Playback levels were measured with a GenRad 1982 sound level meter or a Radio Shack sound level meter calibrated against the GenRad meter. We recalibrated playback levels in the field periodically during each season.

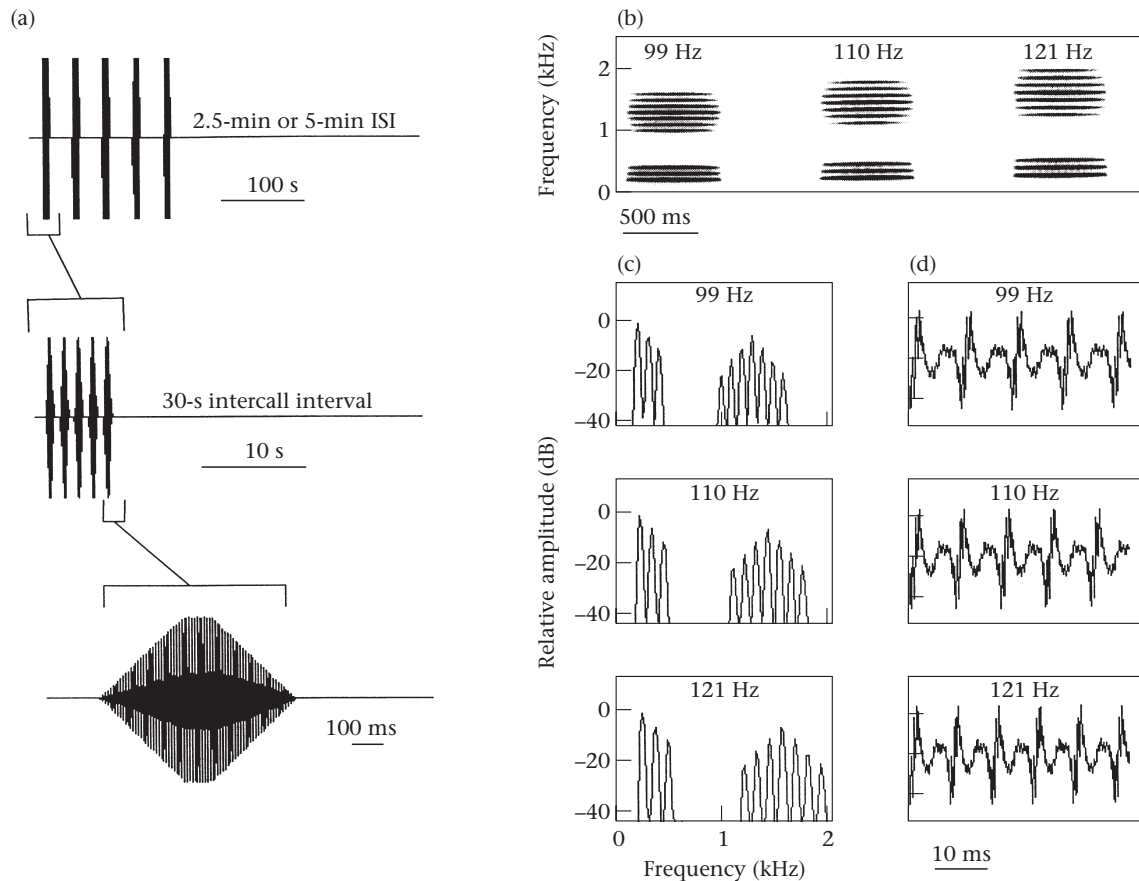


Figure 1. Synthetic bullfrog advertisement calls used as stimuli in this study. (a) Top: one 'stimulus period' with five consecutive five-note calls followed by a 2.5- or 5-min interstimulus interval (ISI). Middle: one five-note call followed by the 30-s intercall interval. Bottom: a single note from the stimulus. (b) Sonograms of a single stimulus note from each of three different stimuli used in playback tests. The fundamental frequencies of the stimuli are (from left to right) 99, 110 and 121 Hz. (c) Power spectra and (d) fine temporal waveforms of the three stimulus notes shown in (b). Note: The 99-Hz stimulus and the 121-Hz stimulus represent a 10% decrease and a 10% increase, respectively, from the fundamental frequency of the 110-Hz stimulus, thus illustrating typical differences between novel and training stimuli in experiment 1.

The stimuli used in this study consisted of synthetic bullfrog advertisement calls (Fig. 1), which we generated at a sampling rate of 20 kHz with 16-bit resolution. Each 'stimulus' consisted of five consecutive five-note advertisement calls separated by 30-s intercall intervals (call duty cycle=0.17). Each call consisted of five identical notes that were 700 ms in duration, had symmetrical linear rise and fall times of 300-ms duration, and were separated by 700-ms internote intervals (note duty cycle=0.50) (Fig. 1a). These temporal properties fall within the range of natural variation for this species (Capranica 1965; Bee & Gerhardt 2001a). Each call note consisted of a series of 10 harmonics (f_2 – f_4 and f_{10} – f_{16}) that had the same starting phase (0°) and were integer multiples of the fundamental frequency (f_1), which was absent from the frequency spectrum (Fig. 1b–d). The dominant frequency was the second harmonic (f_2). All other harmonics were attenuated by 5–20 dB in relation to the dominant frequency. The overall harmonic structure reflected a simplified but typical advertisement call based on acoustic analyses of advertisement calls recorded from the Baskett population. The fifth and final call of the stimulus was followed by a silent 'interstimulus

interval' (ISI) of 2.5 min or 5 min, depending on the experiment. The combination of the stimulus and the ISI was repeated as a continuous loop and, together, they constitute what we refer to as a 'stimulus period' (Fig. 1a, top trace).

During the habituation phase, subjects in both experiments were presented with an acoustically 'size-matched' training stimulus. On the night we tested each subject, prior to the beginning of the playback test, we recorded one or more of its advertisement calls with a Nagra IV-L analogue recorder (tape speed=9.5 cm/s), an HHB PDR 1000 DAT recorder (sampling rate=32 kHz), or a Dell Inspiron 3500 portable computer (sampling rate=22.05 kHz). We used Sennheiser MKH 416T, MKH 815T, MKH 70, or ME 66 directional microphones mounted on a tripod placed 1–2 m from the subject. We haphazardly selected one advertisement call as a representative call from each subject and digitized it with the portable computer as an 8-bit sound file at a sampling rate of 22.05 kHz. We used the waveform display of the Gold-Wave 4.02 software to determine the fundamental frequency of the subject's advertisement call by taking the reciprocal of the average period of 10 repetitions of

the fine temporal waveform of the first note. We used this value to select a synthetic stimulus call with the same (± 1 Hz) fundamental frequency for use as the habituation training stimulus for that particular subject.

Because fundamental frequency is negatively correlated with size in bullfrogs ($r^2=0.81$; see Figure 3a in Bee & Gerhardt 2001a), this procedure acoustically matched the subject and our simulated neighbour for body size. We chose to size-match the subject's own calls for two reasons. First, the aggressive response of male frogs often depends on the perceived size of their opponent based on size-related differences in spectral properties of acoustic signals (Davies & Halliday 1978; Wagner 1989; Bee et al. 1999). Because the most intense agonistic encounters frequently occur between contestants of similar or equal size in a number of animals (Enquist et al. 1990), including bullfrogs (Howard 1978), we preferred that all subjects have the same relative experience of hearing the acoustic signals of a simulated neighbour of similar size. Second, this design allowed us to use a number of different stimuli in playback tests, thereby avoiding potential problems of pseudoreplication and external validity associated with using one or a few stimuli (Kroodsmma 1989, 1990).

We continuously repeated the stimulus period during the habituation phase, which was designed to simulate a new neighbour calling from an adjacent territory. We continued broadcasts of the training stimulus until the subject met an arbitrary criterion of asymptotic response decrement that required the subject to experience three consecutive stimulus periods without responding to the speaker with an encounter call or movement (hereafter 'response decrement criterion'). Males that met this criterion were considered to be familiar with the stimulus, in the sense that presentations of the stimulus no longer elicited aggressive behaviour. After subjects met our response decrement criterion, we immediately began the discrimination phase.

During a playback test, three behavioural responses were scored by one or two observers seated near the pond bank midway between the speaker and the frog's original calling position (usually <5 m from the subject at all times). We counted the number of (1) encounter calls and (2) aggressive movements towards, around, and away from the playback speaker, and (3) we estimated the maximum distance the subject moved towards the speaker (to the nearest 0.5 m). Additional details of behavioural observations have been previously reported elsewhere (Bee & Gerhardt 2001b).

Experiment 1 Protocol: Frequency Discrimination

Experiment 1 tested whether males discriminate between two advertisement calls that differ in fundamental frequency by an amount that falls within the range of between-male differences in our populations (see Figure 3b in Bee & Gerhardt 2001a). During the habituation phase, stimuli were broadcast at 87 dB SPL and were separated by 5-min ISIs. After meeting the response decrement criterion, subjects in this experiment ($N=36$) immediately experienced one of three treatments during

the discrimination phase. These treatments consisted of (1) silence during an interval equivalent to three stimulus periods ($N=12$), (2) continued broadcasts of the training stimulus for three additional stimulus periods ($N=12$), or (3) broadcasts of a novel stimulus for three stimulus periods ($N=12$). Silence and additional presentations of the training stimulus served as controls for response recovery that could occur due to cessation of the training stimulus (Siddle 1990) or to additional broadcasts of any stimulus, respectively.

The novel stimulus differed from the training stimulus by a 10% increase ($N=6$) or decrease ($N=6$) in fundamental frequency and the corresponding differences in correlated spectral components (Fig. 1b–d). We determined the direction of the change randomly for each subject under the constraints that there be equal numbers of increases and decreases over the course of the study and that values not exceed the range of natural variation in fundamental frequency. The gross temporal properties of the training and novel stimuli were identical. We chose a 10% difference because this value exceeds the typical observed magnitude of within-male variation of fundamental frequency, but lies within the range of between-male differences (Bee & Gerhardt 2001a). The novel stimulus was therefore designed to simulate a different male than that simulated by the habituation training stimulus. If male bullfrogs use fundamental frequency (or other correlated properties like dominant frequency) to discriminate between familiar neighbours and unfamiliar males, we predicted that the novel stimulus would elicit significant recovery of the aggressive response compared to our two controls.

Experiment 2 Protocol: Location Discrimination

Experiment 2 tested whether males discriminate between the same stimulus broadcast from two different locations. Our playback protocols differed slightly from that of experiment 1. During the habituation phase, stimuli were broadcast at either 87 or 81 dB SPL and separated by either a 5-min or 2.5-min ISI. Combinations of stimulus intensity and stimulus rate were arranged using a factorial design. After meeting the response decrement criterion during the habituation phase, males were immediately tested in the discrimination phase, in which they experienced a treatment or a control test. We used each of the four possible combinations of stimulus intensity and ISI in a randomly determined order under the constraint that each be used an equal proportion of times within the control and treatment groups in the discrimination phase. Subjects in the control group ($N=8$) heard three additional presentations of the same training stimulus from the original speaker used during the habituation phase. Subjects in the treatment group ($N=12$) heard three additional presentations of the training stimulus from a second speaker placed in a location that was unfamiliar or novel, in the sense that subjects had not previously experienced the stimulus from that location. For males in the treatment group, we used two speakers placed on opposite sides of the subject prior to

the beginning of the test. The second speaker was positioned 6 m away from the subject along the pond bank in the opposite direction (180° relative to the frog) of the habituation phase speaker. The frequency responses of both speakers were within ± 3 dB of each other over the range of frequencies in our stimuli. When possible, we assigned subjects randomly to the treatment or control groups. However, because of the large area of pond bank required to conduct the location test, we preferentially assigned a few males that did not have nearby neighbours on either side to the location treatment group. We chose occasional nonrandom assignments to avoid displacing a subject's adjacent neighbours and placing speakers too close to an existing neighbour. We predicted that if males learned about the location of stimulus origin during habituation training, then aggressive responses should recover when the familiar stimulus was broadcast from an unfamiliar location during the discrimination phase.

Because the environment can degrade and attenuate acoustic signals in complex ways (Wiley & Richards 1978; Richards & Wiley 1980), presenting the training stimulus from a second speaker in a different location has the potential to alter the physical properties of the stimulus along dimensions that territorial residents might use to discriminate between familiar and unfamiliar males. To examine the potential for variation to be introduced into a stimulus by using different speakers in two locations, we broadcast seven stimulus calls ($f_1=95, 100, 105, 110, 115, 120$ and 125 Hz) from each of the speakers used in this experiment. Two speakers were placed 12 m apart in six different locations in three ponds at the Little Dixie site, where experiment 2 was conducted. Locations were selected because they were similar to the areas in which playback tests were completed in terms of both spatial location within a pond and vegetation structure. We recorded the stimuli using an HHB PDR 1000 DAT recorder (sampling rate=32 kHz). The microphone (Sennheiser ME 66) was held just above the water surface at a distance of 6 m from each speaker to simulate the equivalent position of a frog at its calling site during a playback test. Using a Kay DSP Sona-Graph Model 5500, we analysed note duration, rise time, fall time, fundamental frequency and the frequency of each spectral component and its amplitude relative to the dominant frequency for three repetitions of each stimulus from each speaker (see Bee & Gerhardt 2001a for additional details of acoustic analyses). The fundamental frequency and the frequencies of each spectral component of a stimulus did not differ between speakers. The relative amplitude of each of nine harmonics differed by 0–9 dB between speakers (median=2 dB, mode=1 dB), and by 0–3 dB between consecutive recordings of a stimulus broadcast from the same speaker. Our measurements of temporal properties typically varied by 0–12 ms between recordings of a stimulus from the same speaker and also between recordings from different speakers. After testing eight males in the treatment group, we also measured SPLs from each speaker both at the frog's position and at a distance of 1 m from the speaker. Measurements

of stimulus SPLs differed by 2 dB or less between broadcasts from the two speakers.

Data Analyses

To control for potential differences in the initial aggressive responsiveness of males in the treatment and control groups during the discrimination phase, we derived 'percentage of recovery' scores for each response variable by expressing each subject's response during the discrimination phase as a percentage of its initial response at the beginning of the habituation phase. We determined response levels for encounter calls and movements separately by summing the number of times each occurred during both the first three stimulus periods of the habituation phase, as the initial response, and during the three stimulus periods of the discrimination phase, as the recovery response. For maximum approach distance, we used the greatest distance the frog moved towards the speaker during the discrimination phase and the first three stimulus periods of the habituation phase. To be included in the data set, we required that males respond with at least one encounter call during the first stimulus period of the habituation phase.

Because the percentage of recovery scores were correlated within males, and are therefore nonindependent measures of aggression, we used principal components analysis to extract the first principal component from the percentage of recovery scores as a single measure of aggressive response magnitude (e.g. Nelson 1989; Stoddard et al. 1991; Godard & Wiley 1995). If a male did not display an initial response for one of the other response variables during the habituation phase and in response to a stimulus during the discrimination phase, we assigned a percentage of recovery score of zero. Four males tested with the novel stimulus in experiment 1 were excluded from the calculation of principal component scores because of our failure to estimate the distances they moved towards the speaker. No males in experiment 1 displayed movements or approaches towards the speaker during the discrimination phase after failing to do so initially in response to the training stimulus. One male tested with the novel stimulus in experiment 2 displayed movement and approached the playback speaker during the discrimination phase, but failed to do so during his initial response to the training stimulus. Therefore, percentage of recovery scores could not be calculated for these two variables, and the principal component analysis for experiment 2 excluded this individual.

We made between-group comparisons of principal component scores and the percentage of recovery scores using nonparametric statistics because the distributions of the data violated the assumptions of parametric analyses (Hollander & Wolfe 1973; Zar 1984). We analysed the results from each experiment separately. Based on the explicit prediction that habituation would result in stimulus-specific and location-specific response decrements, we tested the null hypothesis that responses in control treatments would be equal to or greater than

those in response to a novel stimulus against the one-tailed alternative that males would respond more aggressively to a novel stimulus. We used a conventional value of $\alpha=0.05$ to determine statistical significance.

RESULTS

In response to broadcasts of a synthetic advertisement call from an unoccupied adjacent territory, male bullfrogs oriented towards the playback speaker, produced encounter calls and approached the speaker using stereotyped aggressive movements. Males often repeatedly charged towards the speaker during the broadcast of the stimulus and returned to their original calling site during a subsequent interstimulus interval. As habituation training proceeded, aggressive responses showed marked decrements, and the majority of males eventually returned to within 0.5 m of their original calling site and resumed producing exclusively advertisement calls. No males gave encounter calls, moved, or approached the speaker during the last three stimulus periods of the habituation training phase. Aggressive response decrements observed in similar playback tests showed several characteristics of response habituation (Bee, 2001; Bee & Gerhardt, 2001b).

Experiment 1: Frequency Discrimination

Males responded more aggressively to the novel stimulus with a different fundamental frequency than to the training stimulus and silence. In terms of principal component scores, the magnitude of the aggressive response differed significantly between the three groups (Kruskal–Wallis ANOVA: $H_2=10.66$, $N=32$, $P=0.0049$; Fig. 2a). Using an all-pairs multiple comparison test based on Kruskal–Wallis rank sums (Hollander & Wolfe 1973), we determined that the novel stimulus elicited significant recovery of the aggressive response compared with both additional presentations of the training stimulus ($P<0.05$) and silence ($P<0.05$). Principal component scores did not differ between the males in the novel stimulus group that heard a 10% increase or 10% decrease in fundamental frequency (Mann–Whitney U test: $U=7$, $N_1=N_2=6$, $P=0.88$). There were also significant differences in the percentage of recovery scores for encounter calls (Kruskal–Wallis ANOVA: $H_2=14.23$, $N=36$, $P=0.0008$), movements ($H_2=12.91$, $N=36$, $P=0.0016$) and maximum approach distance ($H_2=18.46$, $N=32$, $P=0.0001$) (Fig. 2b). Compared with both controls, males responded with more encounter calls ($P_s<0.05$), more aggressive movements ($P_s<0.05$) and approached closer to the playback speaker in response to the novel stimulus ($P_s<0.05$). There were also significant differences in the proportion of males that responded to each test stimulus with encounter calls (chi-square test: $\chi_2^2=9.68$, $P=0.0079$), movements ($\chi_2^2=14.25$, $P=0.0008$) and approaches towards the speaker ($\chi_2^2=17.86$, $P=0.0001$) (Fig. 2c). There were no significant differences in principal component scores or percentage of recovery scores between the two controls.

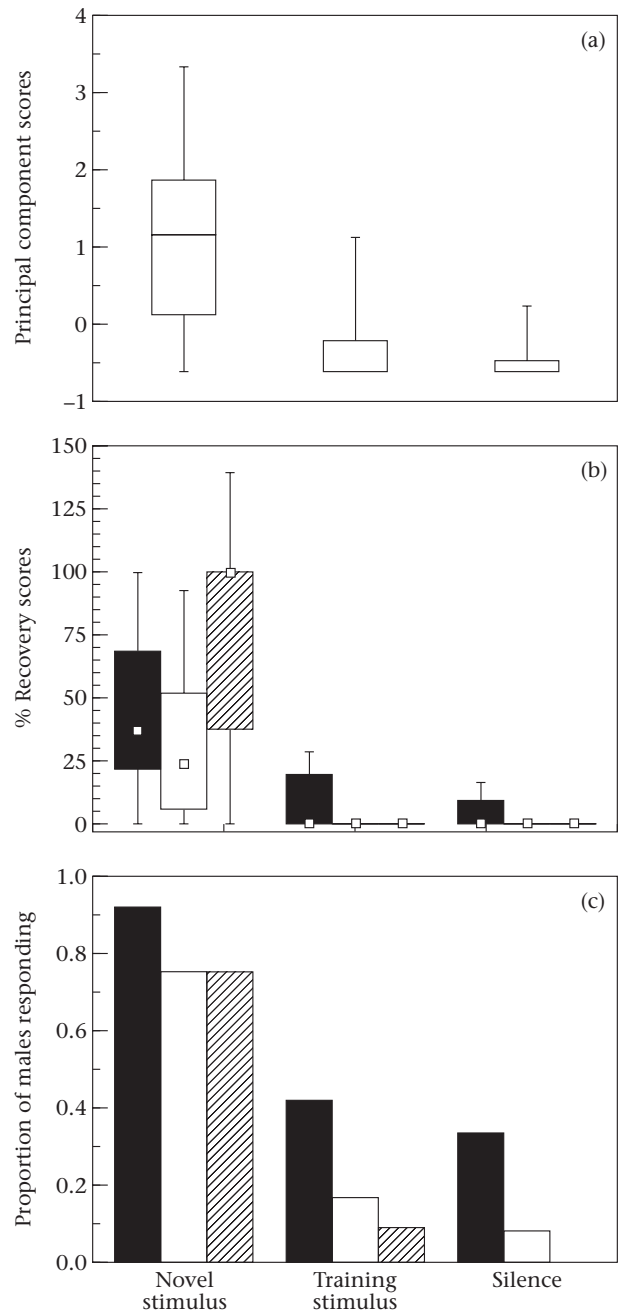


Figure 2. Recovery of aggression during the discrimination phase of experiment 1. (a) Median (bar), interquartile range (box) and range (whiskers) of principal component scores in response to the novel stimulus, the training stimulus, or silence. (b) Median (open square), interquartile range (box) and range (whiskers) of the percentage of recovery scores for encounter calling (■), movement (□) and approach towards the speaker (▨). Principal component scores in (a) were calculated from the percentage of recovery of encounter calls, movement and distance. (c) Proportion of males that responded during the discrimination phase with encounter calls (■), movement (□) and approach towards the speaker (▨).

Experiment 2: Location Discrimination

Broadcasts of the training stimulus from a novel location elicited recovery of the habituated aggressive

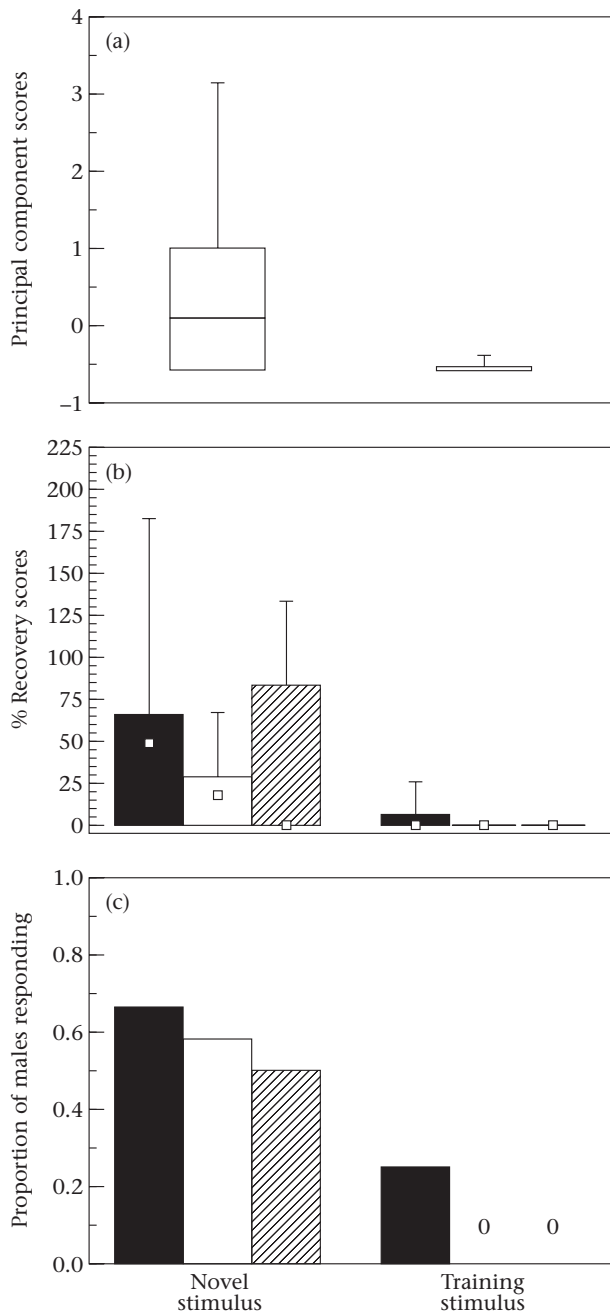


Figure 3. Recovery of aggression during the discrimination phase of experiment 2. (a) Median (bar), interquartile range (box) and range (whiskers) of principal component scores in response to the novel stimulus or the training stimulus. (b) Median (open square), interquartile range (box) and range (whiskers) of the percentage of recovery scores for encounter calling (■), movement (□) and approach towards the speaker (▨). Principal component scores in (a) were calculated from the percentage of recovery of encounter calls, movement and distance. (c) Proportion of males that responded during the discrimination phase with encounter calls (■), movement (□) and approach towards the speaker (▨).

response in the treatment group (Fig. 3). There was a significant difference in the magnitude of the aggressive response based on principal component scores (Mann–Whitney U tests: $U=68$, $N_1=11$, $N_2=8$, $P<0.05$) (Fig. 3a).

Males that experienced the training stimulus in a novel location gave significantly more encounter calls ($U=76$, $N_1=12$, $N_2=8$, $P<0.005$), moved significantly more often ($U=68$, $N_1=11$, $N_2=8$, $P<0.05$), and there was a nonsignificant tendency for males to move closer to the speaker broadcasting the stimulus from a new direction ($U=58$, $N_1=11$, $N_2=8$, NS) (Fig. 3b). We could not detect any differences in the magnitude of recovery to the novel stimulus based on differences in the stimulus intensity or stimulus repetition rate during the habituation phase (Mann–Whitney U tests: NS). There were significant or marginally nonsignificant differences in the proportion of males that responded during the discrimination phase with encounter calls (Fisher's exact tests: treatment versus control, 8/12 versus 2/8, $P=0.0849$), aggressive movement (7/12 versus 0/8, $P=0.0102$) and approaches towards the speaker (5/12 versus 0/8, $P=0.0511$) (Fig. 3c).

DISCUSSION

Frequency Discrimination

Changes of 10% in fundamental frequency (or correlated changes in other spectral properties like dominant frequency) relative to the subject's own calls elicited significant recovery of the habituated aggressive response. These data suggest that male bullfrogs learn about an individually distinct signal property as a result of repeated exposure to a neighbour's calls. Our results also indicate that male bullfrogs discriminate between two stimuli that differ in fundamental frequency by an amount that falls within the range of between-male differences observed in nature. While these findings indicate that fundamental frequency or dominant frequency alone could function as a cue for discriminating among some individuals, we note that the high number of small between-male differences in these properties reported in our companion study (e.g. <4%, Bee & Gerhardt 2001a) suggests that neighbour–stranger discrimination based only on one or both of these properties is likely to be far from perfect. In the absence of additional acoustic or spatial cues, discrimination based solely on fundamental frequency could potentially result in a high number of 'type II' discrimination errors or 'misses', in which an unfamiliar male is mistaken for a familiar one. Neighbour–stranger discrimination in bullfrogs probably also depends on the perception of individual differences in additional call properties. We also point out that our synthetic stimuli did not incorporate any of the variability typically observed within males (Bee & Gerhardt 2001a). Hence, it will be important to determine in future studies whether male bullfrogs can discriminate small differences in fundamental frequency (5–10%) after experiencing a stimulus that incorporates natural levels of intraindividual variation in acoustic properties. Nevertheless, our study suggests that size-related variation in fundamental frequency (or a correlated property) is an important recognition cue mediating neighbour–stranger discrimination in bullfrogs.

Two previous field playback studies have demonstrated that spectral properties of acoustic signals are important

for vocally mediated neighbour–stranger discrimination by territorial songbirds. Brooks & Falls (1975b) found that male white-throated sparrows, *Zonotrichia albicollis*, were more responsive to playbacks of a neighbour's songs in which pitch and duration were changed by 15% compared with the neighbour's unaltered song. Subjects failed to show different responses to changes of only 5%. Changes of 15% in duration alone also failed to elicit an increased response. To elicit increased responsiveness, pitch had to be altered by some threshold amount between 5 and 10%, which corresponded to the amount of natural variation within the songs of individuals (Brooks & Falls 1975b). Nelson (1989) demonstrated that field sparrows, *Spizella pusilla*, responded more to decreases in the pitch of a familiar neighbour's song when the altered pitch fell outside of the typical range of within-male variation, but within the natural range of variation in the species.

Location Discrimination

Results from experiment 2 demonstrate that broadcasts of the training stimulus from a novel location elicit significant recovery of the habituated aggressive response. The implications of this result are the following. First, to the extent to which males can be said to become 'familiar' with a stimulus with repeated exposure, we have corroborated Davis' (1987) original finding by showing that males behaviourally discriminated between familiar sounds broadcast from familiar and unfamiliar locations. Second, and more importantly, we have shown that repeated exposure to synthetic advertisement calls of a simulated new neighbour from an adjacent territory resulted in aggressive response decrements that were specific to the location of the sound source. Male bullfrogs thus potentially habituate to both individually distinct characteristics of a neighbour's calls and to a neighbour's location. We remind readers that the results from this experiment and those of experiment 1 are not directly comparable because we used different training protocols in the two experiments. Hence, a comparison of percentage of recovery scores in the discrimination phases of the two experiments is not an appropriate way to determine the relative influence of changes in frog identity and location on recovery of aggressive responses.

One possible explanation for the apparent location specificity of habituated responses reported here is that broadcasting the stimulus from a different speaker in a different position in the environment caused salient alterations to the acoustic properties of the stimulus. The magnitudes of the differences that we observed in our acoustical analyses of the stimuli from two speakers were small and fell within the range of within-male variation in these call properties (Bee & Gerhardt 2001a, unpublished data). Although a few relatively large differences occurred in the relative amplitudes of the spectral components, we believe these differences are unimportant and did not elicit response recovery for three reasons. First, the auditory periphery of the bullfrog probably does not encode details of the harmonic fine structure of advertisement calls in the frequency domain (Schwartz &

Simmons 1990; Simmons et al. 1992, 1993; Simmons & Ferragamo 1993). Second, the relative amplitudes of some spectral components vary by as much as 5–15 dB between consecutive calls from the same male, and spectral components vary in their presence/absence between calls (Haas 1977; M. A. Bee, unpublished data). Third, results from additional field playback tests based on the habituation/discrimination procedure indicate that synthetic novel stimuli that differ from the training stimulus in the presence/absence and relative amplitudes of various spectral components fail to elicit response recovery during a discrimination phase when speaker location is held constant (Davis 1988; Bee & Gerhardt 2001b). Finally, relatively larger differences in novel stimuli along the dimensions of note duration (70 ms), rise time (30 ms) and fall time (30 ms), compared with those observed between playback speakers (0–12 ms), fail to evoke renewed aggression following habituation training (Bee & Gerhardt, 2001b). Hence, we do not believe that broadcasts from a different location changed the spectral or temporal properties of the training stimulus enough to elicit recovery of the habituated aggressive response.

There have been few studies that have investigated whether the location-specific low level of aggression displayed towards neighbours could result from simply repeatedly experiencing the individual or its communication signals from a particular location. Falls (1969) previously reported what he interpreted as location-specific habituation during field playback tests with Australian magpies, *Gymnorhina dorsalis*. When songs were broadcast every few minutes for several hours, the tendency of birds to approach the speaker decreased, but a subsequent shift in speaker position resulted in renewed responsiveness. Peeke & Veno (1973) reported evidence that aggressive response decrements in territorial three-spined sticklebacks, *Gasterosteus aculeatus*, were specific to the stimulus fish used during habituation training and to the location of stimulus presentation.

Habituation and Neighbour–Stranger Discrimination

Habituation has long been considered a putative psychological mechanism underlying reduced aggression between adjacently territorial neighbours (Weeden & Falls 1959; Brooks & Falls 1975a; Wiley & Wiley 1977; Peeke 1984; Petrinovich 1984; Shettleworth 1998). As a form of perceptual learning, habituation allows animals to learn about the physical properties of a repeated or continuous stimulus (Hall 1991; Shettleworth 1998). As such, habituation could allow territorial residents to learn about the idiosyncratic characteristics of their neighbours or their communication signals (i.e. the stimulus) as a result of repeated exposure across shared territorial boundaries. Several researchers (Falls 1969; Peeke & Veno 1973; Falls & Brooks 1975; Wiley & Wiley 1977) have also suggested that territorial residents might become habituated to the direction or location in which their neighbours are usually found.

In bullfrogs, repeated exposure to a simulated neighbour's advertisement calls resulted in stimulus-specific

and location-specific aggressive response decrements. In another field playback experiment, habituation training produced long-term decrements in aggressive responses that were retained between nights of chorusing and were shown to be specific to the fundamental frequency of the stimulus (Bee & Gerhardt 2001b). Thus, habituation probably plays some role in neighbour–stranger discrimination in bullfrogs, and perhaps other species (Peeke 1984; Petrinovich 1984; Shettleworth 1998). To our knowledge, the present study represents the first demonstration that a territorial animal could habituate to both an individually distinct property of a communication signal and the location from which the signal originates. Our study thus lends support to the general view that habituation to a particular sound from a particular location at least partially mediates the reduced levels of aggression observed between adjacent territorial neighbours. We emphasize that other, more complex forms of information processing may also be involved in learning to recognize a territorial neighbour. For example, recent studies of olfactory cues and individual recognition in rodents demonstrate that familiar odours correspond to representations of familiar individuals and that recognition is more complex than mere familiarity with the odour itself (Johnston & Jernigan 1994; Gheusi et al. 1997). A goal of future studies of vocally mediated neighbour–stranger discrimination should be to determine whether familiar sounds actually evoke mental representations of a particular familiar individual or merely a representation of a particular familiar sound.

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